Role of Adipose Tissue Microvascular Blood Flow in Type 2 Diabetes

By

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Declaration

This work in the present thesis has exclusively been for the use of a Ph.D. in the area of medical research. This thesis contains no material which has been previously been accepted for any higher degree or graduate diploma at the University of Tasmania or any other university. To the best of my knowledge, this thesis contains no material previously published or written by another person, except where due references is made in the text of the thesis.

Donghua Hu

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Preface

The majority of the work presented in this thesis was carried out in the period from August 2014 to March 2018 at the Muscle Research Group, Menzies Institute for Medical Research, University of Tasmania. Some of the data obtained in the present thesis has been presented at scientific meetings or published and are listed below.

Publications

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Scientific meetings

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Abstract

Microvascular blood flow is important to deliver key nutrients (e.g. oxygen, lipids and glucose) and hormones (e.g. insulin) to and remove waste products from adipose tissue. Total adipose tissue blood flow (ATBF) increases after a meal, and this response is impaired in obesity and type 2 diabetes (T2D). Microvascular blood flow is more important than total blood flow for nutrient exchange in many tissues, however it is not known whether microvascular blood flow in adipose tissue is altered by meals or T2D. Chronic exercise training improves microvascular blood flow in skeletal muscle of people with T2D. Whether adipose tissue microvascular responses are similarly improved following exercise training in people with T2D is unknown. The overarching goal of the current thesis is to characterise microvascular ATBF responses to a meal in healthy and T2D subjects, and determine whether these responses are altered by chronic exercise training.

The first aim characterised adipose tissue microvascular blood flow responses in the post-prandial state in healthy people. Adipose tissue microvascular blood flow was measured by contrast-enhanced ultrasound (CEU) at baseline and 1-hour after a mixed meal challenge or an oral glucose challenge (OGC). Adipose tissue microvascular blood volume (MBV) and microvascular blood flow (MBF) increased to a similar extent with both challenges. This increased microvascular perfusion of adipose tissue may improve delivery of key nutrients (e.g. glucose and lipid) from the meal for storage in adipose tissue.

The second aim investigated whether people with T2D have an impairment in adipose tissue microvascular responsiveness following an OGC, and whether systemic inflammation or the metabolic syndrome is associated with an adipose tissue microvascular-linked phenotype. Adipose tissue MBV and MBF post-OGC were markedly impaired in T2D when compared to healthy controls. These impaired microvascular responses in adipose tissue were associated with obesity, insulin resistance, hyperglycaemia and dyslipidaemia, but not systemic inflammation.

The final aim determined whether chronic exercise training restores adipose tissue microvascular blood flow in people with T2D. Adipose tissue microvascular blood flow

was measured by CEU before and after six-weeks (three days per week) of a fully supervised resistance training program. Insulin sensitivity, glycaemic regulation, circulating lipids and body composition were all improved in people with T2D following resistance training. However, these favourable cardio-metabolic outcomes were not associated with a paralleled improvement in adipose tissue MBV and/or MBF.

Collectively, this thesis has demonstrated that a mixed meal or an OGC induces both MBF and MBV increases in adipose tissue in healthy but not people with T2D, and these impairments are not restored by six weeks of exercise training. The dissociation of impaired adipose microvascular blood flow from inflammation, but association with body fat, glycaemic response and lipid handling provides clues about the role of adipose tissue microvascular blood flow in metabolic derangements associated with T2D. In particular, changes in adipose tissue microvascular blood flow in obesity/T2D may affect lipid deposition prior to altering adipose tissue hypoxia and inflammation.

Abbreviations

ACEi	Angiotensin converting enzyme inhibitor
ARB	Angiotensin receptor blocker
ATBF	Adipose tissue blood flow
AUC	Area under the curve
BMI	Body mass index
CEU	Contrast enhanced ultrasound
CRP	C-reactive protein
DBP	Diastolic blood pressure
DEXA	Dual energy x-ray absorptiometry
DM	Diabetes mellitus
DPP4	Dipeptidyl peptidase 4
ERK1/2	Extracellular signal regulated kinase 1/2
FFA	Free fatty acid
GLP-1 RA	Glucagon-like peptide-1 receptor agonist
GLUT4	Glucose transporter 4
GWAS	Genome-wide association studies
HbA1c	Glycated haemoglobin A1c
HDL	High density lipoprotein
HOMA-IR	Homeostatic model assessment of insulin resistance
IKK	IκB kinase
IL-1β	Interleukin 1-beta
IL-6	Interleukin 6
IR	Insulin resistance
IRS	Insulin receptor substrate
JAK-STAT	Janus kinase-signal transducer and activator of transcription
JNK	Jun NH2-terminal kinase
LDF	Laser Doppler flowmetry
LDL	Low density lipoprotein
L-NMMA	N_G-monomethyl-l-arginine
MBF	Microvascular blood flow
MBV	Microvascular blood volume
MCP-1	Monocyte chemoattractant protein - 1
MMC	Mixed meal challenge
NF-κB	Nuclear factor-κB
NO	Nitric oxide
OGC	Oral glucose challenge
PET	Positron emission tomography
PI3K	Phosphatidylinositol-3-kinase
PKC	Protein kinase C
QUICKI	Quantitative insulin sensitivity check index
RT	Resistance training

SBP	Systolic blood pressure
SGLT2	Sodium-glucose cotransporter 2
SOCS	Suppressor of cytokine signaling
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TCF7L2	Transcription factor 7-like 2
TG	Triglycerides
TNF-α	Tumour necrosis factor alpha
VCAM-1	Vascular cell adhesion molecule -1

Chapter 1: Thesis Introduction

1.1 Preface

Type 2 diabetes (T2D) is a chronic disease affecting more than 300 million people globally. It is a chronic, progressive disease characterized by elevated levels of blood glucose. Long term elevated blood glucose can lead to complications in many parts of the body and can increase mortality and morbidity. T2D also causes a large economic burden on health-care systems and the global economy. Insulin resistance (IR) is suggested to develop as a result of obesity, predisposing to the onset of T2D. Obesity is initiated by several factors such as physical inactivity, excess calories and genetic predisposition. Obesity is also strongly associated with a state of chronic low-grade inflammation in adipose tissue, which may also contribute to IR. Adipose tissue expansion (hypertrophy) is thought to result in insufficient blood flow, leading to adipocyte hypoxia and the latter is thought to promote inflammation. The current management of T2D involves a combination of lifestyle modifications such as exercise, reduced energy intake and use of medications designed to lower glycemia. Recently, resistance training (RT) has been recommended for T2D management. While benefits for glucose control and insulin sensitivity in skeletal muscle have been investigated after RT, it is not known whether this involves augmentation of adipose tissue microvascular blood flow. Therefore, this project aims to i) characterise adipose tissue microvascular blood flow responses in the post-prandial state in healthy subjects, ii) investigate whether adipose tissue microvascular responses are reduced in people with T2D in the post-prandial state, and iii) determine whether RT alters post-prandial adipose tissue microvascular responses in people with T2D.

1.2 Obesity and type 2 diabetes

1.2.1 Diabetes mellitus

Diabetes mellitus (DM), which has been linked with a range of health complications, is considered a global epidemic in the 21st century [1]. According to the International Diabetes Federation, the worldwide prevalence of DM was approximately 415 million in 2015 and the number is predicted to increase to 642 million by the year 2040 [2]. According to Diabetes Australia, about 1.7 million people in Australia had DM in 2017. This includes diagnosed diabetes (1.2 million known and registered) and undiagnosed DM (up to 500,000) [3]. In Australia, the annual cost of DM has been estimated at approximately $14.6 billion [3]. Also, elevated blood glucose levels over long periods of time can lead to dysfunction in both small and large blood vessels [4]. Microvascular dysfunction can lead to complications, for example blindness (retinopathy), nerve disease (neuropathy) and kidney disease (nephropathy). Macrovascular dysfunction can lead to cardiovascular disease (e.g. heart attack) [1, 5]. These vascular complications are a major contributor to increased mortality and morbidity, and a large financial burden to health-care systems globally [6].

DM is a progressive, chronic metabolic disorder, characterised by long term high blood glucose levels, termed hyperglycaemia [1]. DM, including type 1 diabetes (T1D), type 2 diabetes (T2D) and gestational diabetes for women, are complex metabolic disorders that result from defects in insulin secretion, action, or a combination of both [7]. T1D makes up approximately 10% of all cases of DM and is caused by an autoimmune destruction of the insulin-producing β cells of the pancreas, abolishing insulin section. It is still not known what causes this auto-immune reaction [8]. T2D, which often begins with insulin resistance (IR), is a condition in which cells fail to respond to insulin properly and makes up about 80-90% of cases of DM [9]. Gestational diabetes, which is defined as diabetes diagnosed during pregnancy, usually goes away after the baby is born but increases the risk of T2D in these women later in life [10].

T2D is a chronic metabolic disorder that is characterized by hyperglycaemia, IR, and relative lack of insulin production [1, 11]. According to WHO diabetes diagnostic criteria, clinical diagnosis of T2D include: fasting blood glucose level ≥7.0 mmol/L

(126mg/dl) (on two separate times) and/or glycated haemoglobin (HbA1c) ≥6.5% (on two separate times) and/or 2-hour postprandial blood glucose ≥11.0 mmol/L (200 mg/dl) (in response to an oral 75g glucose tolerance test on two separate times). The aetiology of T2D is multifactorial, including family history, IR, physical inactivity and obesity, but specific mechanisms are not fully understood [12]. However, the development of IR is thought to be a universal precursor for developing T2D [13].

1.2.2 Insulin resistance and T2D

IR (observed in pre-diabetic states) precedes the onset of T2D, and plays a pivotal role in the development and pathogenesis of T2D [14]. IR often occurs 10 to 15 years prior to the development of T2D, and it is accompanied by compensatory hyperinsulinaemia [14]. Insulin, which is from the Latin word, *insula* (meaning island), is a peptide hormone secreted from β cells of the pancreatic islets [15]. It has important effects on the metabolism of carbohydrates, fats and protein. For example, insulin not only reduces blood glucose by improving glucose uptake in skeletal muscle, adipose tissue and heart, but it also inhibits glucose production in liver [16] and decreases lipolysis [17].

In order to promote glucose uptake in healthy subjects, insulin causes the movement of glucose transporters from intracellular membranes to the cell surface [18]. The insulin receptor substrate (IRS) proteins play a key role in insulin signaling. In fact, glucose transporter 4 (GLUT4) is highly expressed in adipose tissue and skeletal muscle and it is an insulin-responsive member of the glucose transporter family [19]. It is activated by binding of insulin to its receptor, which activates its tyrosine kinase activity and phosphorylates IRS proteins (particularly IRS-1), in turn activating phosphatidylinositol-3-kinase (PI3K) and other downstream effectors [20, 21]. PI3K signals through the serine-threonine kinases Akt2 [22, 23] and protein kinase C (PKC) isoform λ(PKCλ) [24] to control GLUT4 translocation, leading to glucose transport into the cell and stimulation of glycogen synthesis [25]. However, disruption to any part of this pathway can result in IR.

IR affects the ability of tissues to respond to insulin which is secreted into the circulation in the post-prandial state [26]. During the early stages of IR, insulin

produces a less than expected biologic effect and, in turn, reducing whole body glucose disposal [27]. The increased postprandial glucose level drives up insulin secretion further, leading to hyperinsulinaemia [27]. Consequently, IR results in pancreatic "stress", pancreatic β cell dysfunction and eventually elevated blood glucose levels. During the later stages of T2D, the pancreas is not able to produce sufficient amounts of insulin and during these circumstances exogenous insulin injection is required to manage blood glucose levels [27].

IR arises from impaired insulin action in metabolic organs and tissues such as skeletal muscle, liver, and adipose tissue [28]. In skeletal muscle, insulin plays an essential role in stimulating glucose uptake and metabolism [29, 30]. IR is associated with reduced insulin-mediated glucose transport into muscle and muscle glycogen synthesis [30-32]. In the liver, IR is characterized by impaired insulin-mediated suppression of gluconeogenesis in the fed state [33]. Hepatic IR is also associated with non-alcoholic fatty liver disease which is a major risk factor for the development of T2D [34-36]. In adipose tissue, IR is manifested as impaired insulin-stimulated glucose transport and impaired inhibition of lipolysis [37, 38].

1.2.3 Risk factors for developing T2D

T2D mostly results from the interaction between genetics, physical inactivity and elevated central fat. Indeed, T2D has a strong genetic link [39]. People with a family history of T2D have a 3 to 4-fold higher risk of developing diabetes than those without family history of T2D [40, 41]. In particular, 39% of T2D patients have at least one affected parent [42] and the lifetime risk for a first-degree relative of a patient with T2D is 5 -10 times higher than that of age- and weight-matched subjects without a family history of T2D [43]. This strong family history of the disease suggests the involvement of genetic factors for diabetes development. A number of genome wide association study (GWAS) have been conducted in diverse populations demonstrating linkage signals in chromosomes of people with T2D, and have successfully confirmed about 75 sensitivity loci correlated to T2D [44]. An example is genes that encode proteins such as TCF7L2 (transcription factor 7-like 2, the strongest T2D locus identified to date) that cause alterations in several pathways, leading to T2D [45-47].

In addition to genetics, physical inactivity plays a key role in the development of T2D [45, 46]. A sedentary lifestyle reduces insulin sensitivity in target tissues (liver, muscle and adipose tissue) promoting progression to T2D. In contrast, increased levels of physical activity may ameliorate IR by improving insulin action and vascular function [45, 47]. Studies have shown that low levels of physical activity are involved in the development and progression of IR and T2D [48, 49]. Television watching represents a major sedentary lifestyle habit in the United States for example. Adult men spend on average about 29 h per week watching television, and adult women 34 h per week [50]. Hu and colleagues followed a total of 1,058 participants and examined the relationship between television watching time and the incidence of T2D. They found that time spent watching television was associated with a significantly higher risk of T2D [51]. Television watching is associated with obesity and weight gain, probably because of less energy expenditure (i.e., less physical activity) and higher energy intake [50]. Higher levels of physical activity are linked to decreased risk factors for developing IR and T2D [52] which is at least in part due to weight loss.

Central obesity is a major risk factor for developing T2D [53]. Not all individuals with T2D are obese and many obese individuals do not have diabetes, but the majority of T2D individuals have elevated body weight, in particular fat located in the abdominal area [54].

1.2.4 Obesity and type 2 diabetes

Obesity is defined as a chronic condition caused by excessive fat accumulation resulting from an energy imbalance [55]. It can be measured simply using body mass index (BMI). It is calculated from the weight-for-height ratio and is widely used for classifying overweight and obesity in adults. BMI is defined as: underweight: below 18.5 kg/m^2; healthy weight: 18.5 to 25 kg/m^2; overweight: 25 to 30 kg/m^2; obese: over 30 kg/m^2 [56]. Overweight and obesity are huge public health challenges worldwide now [55]. In 2013, 36.9% of men and 38% of women were overweight/obese adults, and 23.8% of boys and 22.6% of girls were children and adolescents [57]. According to Australia Obesity Statistics in 2017, 63.4% of Australian adults (11.2 million people) are overweight or obese [58]. For middle-aged men the problem is magnified with 75 - 80% being overweight. Also, 25% of children are classified as overweight in this

country. Moreover, there is a strong association between BMI and risk of developing T2D [59, 60]. For instance, the prevalence of diabetes increased from 2% in people with a BMI of 25 to 29.9 kg/m^2, to 8% in people with a BMI of 30 to 34.9 kg/m^2, and finally to 13% in people with a BMI more than 35 kg/m^2 [61]. Each kilogram of weight gained annually over a period of 10 years is associated with a 49% increase in risk of developing T2D in the subsequent 10 years [11]. In contrast, each kg of weight lost annually over 10 years was correlated with a 33% lower risk of diabetes in the subsequent 10 years [11].

Although overall obesity confers a significant threat to individual health, central obesity has been recognised as an independent higher risk factor for developing metabolic disorders and cardiovascular diseases [62, 63]. Indeed, central obesity is a risk factor for IR, T2D, hypertension and increased cardiovascular morbidity and mortality [64]. Also, central obesity plays a key role in development of IR in various tissues, including liver, skeletal muscle and adipose tissue, leading to T2D [65]. How central obesity causes IR is not fully understood. However, elevated free fatty acids (FFAs) that are released from the expanded adipose tissue are strongly implicated in the development of peripheral (skeletal muscle) and hepatic IR [66]. Also, adipose tissue *per se* can develop IR which is characterised by reduced insulin-stimulated glucose disposal and impaired lipogenesis which contributes to elevated circulating FFAs.

1.2.5 Obesity and insulin resistance in adipose tissue

It has been suggested that IR and impaired insulin action in adipose tissue occur much earlier than glucose intolerance (in fact, a long time before glucose intolerance develops) [67]. Adipose tissue consists of adipocytes (cells that store fat), as well as cells of the stromal vascular fraction which includes pre-adipocytes (that can differentiate into mature adipocytes), vascular smooth muscle and endothelial cells (comprising blood vessels) and macrophages [68]. The main role of adipose tissue is to store excess calories in the form of triglycerides, and it was thought to be involved only in body lipid and energy homeostasis [69]. Recently, it has become more clear that, in addition to lipid storage, adipose tissue is an important secretory organ, which produces adipokines

and inflammatory cytokines [70]. Disturbances in lipid metabolism (especially FFAs) and adipose tissue inflammation might directly contribute to the onset of IR [71].

Indeed, central obese people are characterized by elevated plasma FFA levels. It has been established that elevated plasma FFAs play an important role in the pathogenesis of IR [72, 73]. With weight gain, FFAs are elevated in obese people and activate protein kinases such as PKC, Jun NH2-terminal kinase (JNK), and the inhibitor of nuclear factor-κB (NF-κB) kinase-β (IKKβ) in a number of tissues. This can lead to insulin signalling being impaired by these kinases, via an increase in the inhibitory serine phosphorylation of IRS (a key mediators of insulin receptor signalling), leading to IR [74]. Studies showed that acute elevations of plasma FFA levels (by intravenous infusion of heparinized lipid emulsions) lead to IR [66, 75]. The FFA-induced IR developed after 2 hours and disappeared about 4 hours after normalization of plasma FFA. This effect was dose dependent IR [66, 75]. Chronically elevated plasma FFA levels are also strongly implicated in the development of IR. Santomauro and colleagues have shown that acipimox (250 mg) (a long-acting nicotinic acid analogue that lowers plasma FFA levels by inhibiting lipolysis) decreased fasting plasma FFA concentrations (by 60-70%) and plasma insulin (by about 50%) in lean and obese people. This suggests that lowering plasma FFA concentrations can decrease IR/hyperinsulinaemia and improve oral glucose tolerance in lean and obese people with and without T2D [76].

Obese conditions are also often associated with a state of low-grade chronic inflammation. Adipose tissue expansion is thought to result in insufficient blood flow and hypoxia to the adipocyte, triggering production of inflammatory cytokines, which affect insulin signaling, resulting in IR [77]. During the last several decades, experimental, epidemiological, and clinical studies have shown causal links between inflammation and the development of IR and T2D [78, 79]. Activation of inflammatory pathways in hepatocytes might induce both local [80] as well as systemic IR [81]. The role of inflammatory markers in development of IR is further detailed in section 1.3.

1.2.6 Raised plasma FFAs causes insulin resistance

The usual cause of obesity is an inbalance between energy intake and output. Generally, lipids are stored as triglycerides in adipose tissue (e.g. after meal ingestion) and to release FFAs for utilization by oxidative tissues (e.g. skeletal muscle, heart, and liver) in times of energy demand (e.g. fasting, exercise) [82]. Adipose tissue may occupy 50 % of the whole-body weight in an adult, and this tissue can provide more than 85% of the total stored energy [83]. However, when adipose tissue cannot meet the demand for storing excessive energy, triglycerides are accumulated as ectopic fat (defined by excess adipose tissue in sites not classically in adipose tissue storage) and excessive FFAs are released into the circulation which also impair insulin signalling in muscle and the liver, leading to IR [84, 85].

Plasma FFA levels are about three-fold higher in abdominally obese adults when compared to non-obese adults [86, 87]. Skeletal muscle accounts for about 80 % of postprandial insulin-stimulated glucose disposal. However, skeletal muscle is also a major site of FFA utilization [88, 89]. Studies in obese humans and high fat-fed rats have revealed an enhanced transport of FFAs into skeletal muscle which is associated with an increased intramyocellular triglycerides content [90, 91]. FFAs in skeletal muscle are stored in the form of intramyocellular triacylglycerol and represent an important source of energy [92]. However, excessive accumulation of FFAs suppress insulin-stimulated glucose uptake at the level of glucose transport and/or phosphorylation [93, 94]. The possible mechanism is that elevated intramyocellular diacylglycerol and long-chain acyl-CoA, activate PKC, and decrease tyrosine phosphorylation of IRS-1, and this is thought to be central to the pathogenesis of IR-associated diseases such as T2D [35, 92, 95]. Also, elevated intramyocellular lipids in skeletal muscle have been demonstrated to activate PKC and the IκB kinase (IKK)/NF-κB signaling pathways, which affect downstream action of the insulin signaling pathway, leading to IR [17]. For the liver, increasing hepatic FFAs flux, which increase hepatic glucose production, have been thought as a main driving force for systemic IR [96, 97]. Studies have shown that higher FFAs cause hepatic IR, lead to hepatic accumulation of diacylglycerol and activate two serine/ threonine kinases (PKC-δ and IKK-β), which play a key role in developing T2D [96-98].

The transport of triglycerides and FFAs to and out of the tissue involves specialized transport mechanisms including delivery and removal from adipose tissue by the

vasculature [99]. Increased postprandial plasma triglyceride and FFA is associated with reduced adipose tissue blood flow (ATBF) [100]. ATBF has been suggest to impact on lipid clearance [101]. This suggests that ATBF potentially plays an important role in lipid metabolism [83]. ATBF will be discussed more detail later.

1.3 Inflammation in adipose tissue

1.3.1 Inflammation and obesity

Inflammation is a physiological reaction necessary to restore homeostasis and characteristics of inflammation are raised numerous white blood cells or elevated levels of pro-inflammatory cytokines in the circulation or in tissue [102]. In general, inflammation is required for organ remodelling, tissue repair, wound healing and immunity against infections [16]. However, in overweight and obese individuals, adipose tissue releases inflammatory cytokines, which are associated with the pathogenesis of a number of chronic diseases, such as IR, cardiovascular disease and T2D [103].

In insulin sensitive individuals, adipose tissue has capacity to increase its size dramatically [104]. Sufficient oxygen supply plays a determinant role in maintaining normal adipose tissue function [105]. During obesity, adipose tissue expansion can occur in two ways, via hypertrophy (increase in adipocyte size) or via hyperplasia (increase in adipocyte number), with the former being more metabolically detrimental [106]. This is in part due to the adipocyte increasing in cell size while there is a concomitant decrease in microvascular density, which may limit oxygen supply, leading to hypoxia [107, 108]. Local tissue hypoxia may occur at the early stages of adipose tissue expansion and limit normal adipose tissue function [84]. In this hypoxic environment, macrophages are activated to release inflammatory cytokines [109, 110]. Studies have suggested that the oxygen partial pressure in adipose tissue is low during obesity in both humans and animals [111-115] and may be an important link to hypoxia-driven chronic inflammation in obesity [111].

Moreover, due to adipose tissue expansion, adipose tissue becomes dysfunctional [116]. During obesity, when adipose tissue becomes dysfunctional it can lead to exaggerated

release of FFAs and pro-inflammatory cytokines leading to ectopic lipid deposition, which together contribute to IR and T2D [117] . In one study, three months of high fat feeding of mice caused low grade hepatic inflammation [118]. This showed that the inflammatory state was linked to the high fat diet. FFA may be one of candidates because FFA and inflammation are both elevated in most obese subjects in the post-prandial state [119, 120] . In addition, acutely raised plasma FFA not only resulted in peripheral and hepatic IR, but also activated the pro-inflammatory NF-κB pathway, leading to increased production of pro-inflammatory cytokines [97, 121].

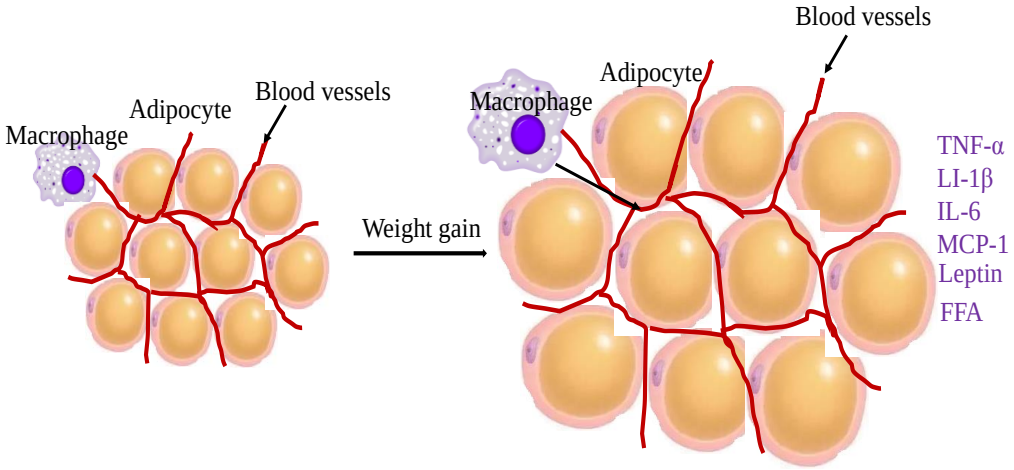

Figure 1.1: Summary of inflammatory cytokines develop during the obese status: adipose tissue hypertrophy, macrophage infiltration and changed adipokine secretion. During weight gain, adipose cell increases its size, which leads to adipose tissue hypertrophy, macrophage infiltration and high levels of pro-inflammatory markers.

1.3.2 Inflammation and insulin resistance

Adipose tissue is a highly dynamic endocrine organ, secreting a large number of adipokines such as leptin, adiponectin and inflammatory cytokines [122-124]. Inflammatory cytokines play a key role in the development of IR via various molecular pathways [125, 126]. As discussed above, during obesity it is thought that as the adipocyte undergoes hypertrophy, insufficient microvascular blood supply to the adipocyte leads to hypoxia, macrophage recruitment and conversion of macrophages from an inactive state (M2) to an active state (M1) in which they are reported to release

tumour necrosis factor alpha (TNF-α), interleukin 6 (IL-6), interleukin-1 beta (IL-1β) and monocyte chemoattractant protein-1 (MCP-1) [85, 127, 128] (Figure 1.1). Moreover, TNF-α, IL-1β, IL-6, C-reactive protein (CRP), MCP-1 and soluble vascular cell adhesion molecule-1(sVCAM-1) are possibility associated with obesity or T2D [129-135].

Adipose tissue inflammation may contribute to whole-body IR and T2D via the endocrine effects of inflammatory cytokines secreted by adipose tissue on insulin sensitivity in various tissues, particularly in skeletal muscle and liver [125, 126]. Six inflammatory markers have been found to be associated with IR. As discussed below, there are several pathways of how inflammatory markers may be coupled to impaired insulin signalling in adipocytes, leading to IR and T2D (Figure 1.2).

1.3.3 Tumour necrosis factor alpha (TNF-α)

TNF-α is a pro-inflammatory cytokine produced by various cell types such as adipose cells, although predominantly by macrophages and lymphocytes [136]. The primary role of TNF-α is in the regulation of immune cells and make up the acute phase reaction [137]. TNF-α increases have been identified in adipose tissue and increased during obesity and it has been shown to interfere with insulin signalling [138]. Mice lacking TNF-α are protected from obesity-induced IR [139]. Hotamisligil and colleagues have shown that increased TNF-α expression in adipose tissue of obese humans is correlated with the degree of hyperinsulinaemia, while TNF-α expression is decreased with weight loss, suggesting a potential mechanistic role in human obesity-induced IR [140]. It has been suggested that TNF-α inhibits insulin signal transduction and it may also decrease insulin secretion [141]. Studies have shown that TNF-α impairs skeletal muscle insulin signalling and reduces whole body glucose uptake in healthy animals and humans [142, 143]. TNF-α also causes an increase in FFA release by adipocytes, leading to increased levels of circulating FFAs, which can reduce insulin signalling in other tissues [144].

TNF-α is an adipose tissue-derived pro-inflammatory cytokine that causes IR by improving adipocyte lipolysis and enhancing the serine/threonine phosphorylation of IRS-1[67, 79]. Actually, TNF-α triggers a broad spectrum signaling cascade that results in the activation of various transcriptional pathways such as NF-κB and JNK [145, 146].

Once NF-κB and JNK are activated, they phosphorylate serine 307 in IRS-1 which results in the impairment of IR-mediated tyrosine phosphorylation of IRS-1 [145].

1.3.4 Interleukin-1 beta (IL-1β)

IL-1β plays a key role in regulating the body's inflammatory response and it is produced during infection, injury, and antigenic challenge [147]. The macrophage produces most of the IL-1β in the body, but epidermal, epithelial, lymphoid and vascular tissues also synthesize IL-1β [148-150]. Mandrup-Poulsen and colleagues demonstrated that IL-1β impaired β-cell function [151, 152]. In addition to impaired β-cell function, IL-1β was suggested to cause β-cell death, which was potentiated by the cytokines such as interferon-γ (IFN-γ) and TNF-α [153, 154]. The possible mechanism is that IL-1β principally affects the activation of mitogen-activated protein kinases (MAPK) and NF-κB on expression of the encoding gene in the process of β-cells death [148, 155].

IL-1β expression is highly upregulated in pancreatic islets of patients with T2D [156-158]. It plays a key role in developing IR by attenuating insulin signalling in peripheral tissues and macrophages, resulting in insulin sensitivity reduction of β-cells and possibly impaired pancreas action [159, 160]. IL-1β was shown to dramatically increase suppressor of cytokine signalling - 3 (SOCS-3) protein expression, which can impair insulin signalling by binding to the insulin receptors and suppress their capacity to phosphorylate IRS proteins [161]. Moreover, SOCS proteins are able to bind directly to IRS proteins, which results in IRS degradation [162, 163]. Indeed, it has been suggested that IL-1β decreased insulin-stimulated glucose transport in adipocytes primarily by supressing IRS-1 levels expression via a reducing IRS-1 mRNA [164].

1.3.5 Interleukin 6 (IL-6)

IL-6 is secreted by multiple tissues such as skeletal muscle and liver, and particularly in adipose tissue, which secretes about 30% of IL-6 in healthy individuals [125, 165]. The major function of IL-6 is to activate immune response during infection and after trauma, particularly burns or other tissue damage, resulting in inflammation [166]. IL-6 is implicated as a predictor or pathogenic mediator of cardiovascular disease and IR

[167, 168]. IL-6 secretion is increased in obese subjects and may contribute to regulation of insulin action [169]. IL-6 is suggested to be a typical pro-inflammatory cytokine because its expression is enhanced in adipose tissue during weight gain, especially from the visceral depot [170]. Abdominal adipose tissue produces 3-fold higher IL-6 than subcutaneous adipose tissue, suggesting that IL-6 produced by abdominal adipose tissue is a potential factor for developing of IR [171]. Also, plasma IL-6 levels are elevated in people with T2D, particularly in those with features of insulin resistance syndrome and the elevated circulating level of IL-6 is an independent predictor of T2D [155, 172].

IL-6 is recognized as causing IR by decreasing the expression of GLUT-4 and IRS-1 [125]. Also, these effects are influenced by the stimulation of the Janus kinase-signal transducer and activator of transcription (JAK-STAT) signalling pathway and enhanced the expression of SOCS family (e.g. SOCS-3), inhibiting insulin receptor signal transduction[173]. Interestingly, IL-6 has been suggested to be an interleukin that acts as both a pro-inflammatory cytokine and an anti- inflammatory cytokine [174, 175]. Indeed, there are a number of studies showing that IL-6 has anti-inflammatory effects [176] and that it may even play an important role in improving insulin sensitivity in skeletal muscle [177]. Regarding IL-6, the pro- versus anti- inflammatory properties of IL-6 are context dependent (acute versus chronic, skeletal muscle or adipose tissue origin) [174]. Studies have shown that IL-6 levels increased after acute exercise and during exercise [178, 179]. Also, acute IL-6 treatment enhances insulin sensitivity in humans [177].

1.3.6 C-reactive protein (CRP)

CRP is synthesized by the liver and is induced by pro-inflammatory cytokines including IL-6 and TNF-α [180, 181]. The main function of CRP is in the innate immune system and it is the first acute-phase protein to be described. It is a sensitive systemic indicator of inflammation and tissue damage [182, 183]. Moreover, increased CRP levels have been associated with endothelial dysfunction and vascular inflammation [184]. It has been suggested that high circulating levels of CRP are a marker for cardiovascular disease risk and circulating CRP has also been significantly correlated with IR [183, 185-187].

In most human studies, CRP is detected by using high-sensitivity assays and is identified as high-sensitivity CRP (hs-CRP)[188]. Studies have shown that elevated serum hs-CRP was dose-dependently associated with an increased degree of IR [187, 189, 190]. Hs-CRP may cause IR by causing IRS-1 phosphorylation at Ser307 and Ser612 through JNK and ERK1/2, respectively, resulting in impaired insulin-induced glucose uptake, GLUT4 translocation, and glycogen synthesis by the IRS-1/PI-3K/Akt/GSK-3 pathway [191, 192]. Many studies have demonstrated that people with elevated levels of CRP have a high risk of T2D [193, 194]. For example, a recent study showed that one-third of T2D cases are associated with elevated serum CRP [195]. However, a meta-analysis has suggested that hs-CRP may not always be an independent risk factor for developing T2D [196].

1.3.7 Monocyte chemoattractant protein-1 (MCP-1)

MCP-1 (also called CCL2) is a pro-inflammatory chemokine produced by adipocytes, macrophages, smooth muscle, and endothelial cells [79, 197]. MCP-1 is a member of the C-C chemokine family and plays a key role in regulating migration and infiltration of monocytes/macrophages [198]. MCP-1 is associated with various diseases such as multiple sclerosis[199], rheumatoid arthritis [200], atherosclerosis [201], IR and T2D [202]. MCP-1 attracts monocytes to the inflammatory area of vascular endothelial space, which causes atherogenesis in large arteries and obesity-stimulated macrophage infiltration in fat tissue [203].

Adipose tissue macrophage infiltration is close linked with IR and vascular endothelial dysfunction in obese individuals [204]. The expression of MCP-1increases during obesity, especially in visceral fat areas, and might play an important role in developing IR, particularly in the liver [197, 205]. Studies have shown that MCP-1 is overexpressed in diet-induced IR and obese mice (*ob/ob* and *db/db* mice) compared with their lean controls, MCP-1 might contribute to the development of IR in adipose tissue and cause adipocyte de-differentiation [197, 202, 206]. Also, MCP-1 overexpression by adipose cells leads to hepatic steatosis and IR in liver, muscle and adipose tissue [197, 202, 206]. Moreover, circulating MCP-1 was significantly increased in people with IR status or T2D [207-209]. It has been shown that MCP-1, via activation of extracellular signal

regulated kinase (ERK) 1/2 pathways, and causes an increase in the expression of amylin in pancreatic β cells and IR, promoting development of T2D [210, 211].

1.3.8 Soluble vascular cell adhesion molecule-1 (sVCAM-1)

VCAM-1 is expressed mainly by endothelial cells, smooth muscle cells, and tissue macrophages [212, 213]. Soluble forms (sVCAM-1) have been identified in plasma [214]. VCAM-1 expression is increased by several inflammatory molecules like TNF-α and IL-1 [215]. sVCAM-1 plays a key role in accelerating atherosclerosis by facilitating the attachment of inflammatory cells to the vascular endothelial wall and promoting their subsequent migration through the endothelium [216]. The resulting inflammatory response, injury, and stiffening of the vascular wall may result in impaired blood flow regulation in organs and tissues [216].

Increased plasma concentrations of sVCAM-1 are found with increasing adiposity, which demonstrated that increased adipose tissue mass may be correlated with increased endothelial activation [217]. VCAM-1, which is released from adipose cells, may play an important role in recruiting immune cells into adipose tissue [218]. Elevated concentrations of sVCAM-1 is also linked to dyslipidaemia, cardiovascular disease, IR and T2D [135, 219-222]. Indeed, epidemiological studies have shown that elevated sVCAM-1 levels signify endothelial dysfunction and independently predict the risk of future T2D [223-225]. Although the molecular mechanisms for the relationship between sVCAM-1 and T2D are unclear, it has been postulated that endothelial dysfunction induced by sVCAM-1 may lead to IR and thereby facilitate progression to T2D [226, 227].

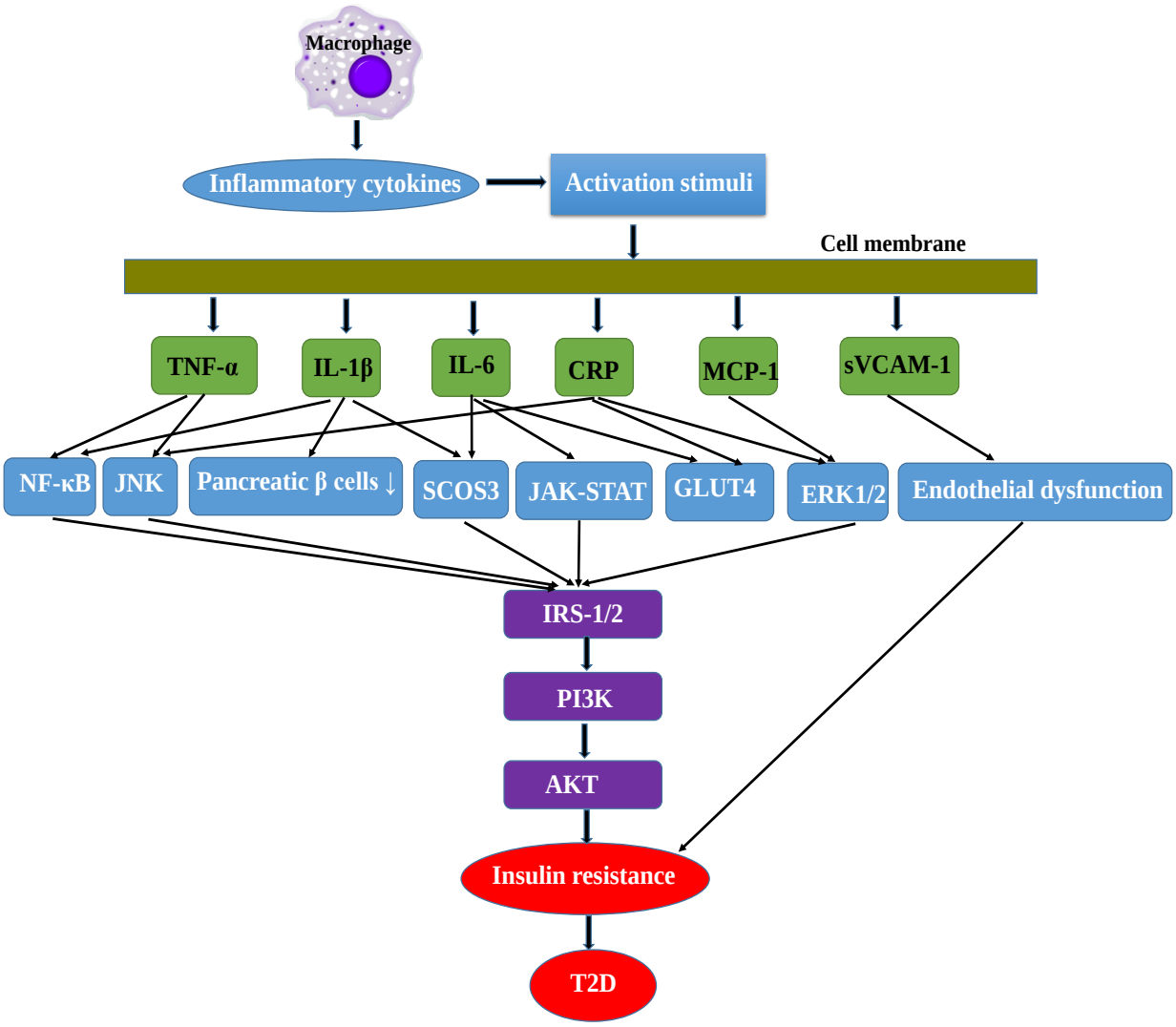

Figure 1.2: Summary of inflammatory pathways linking inflammation to insulin resistance and T2D. NF-κB: nuclear factor κB; JNK: Jun NH2-terminal kinase; SCOS3: suppressor of cytokine signaling-3; JAK-STAT: Janus kinase-signal transducer and activator of transcription; GLUT-4: glucose transporter-4; ERK1/2: extracellular signalregulated kinase 1/2; IRS-1/2: insulin receptor substrate-1/2; PI-3K: phosphatidylinositol-3-kinase.

1.4 ATBF

1.4.1 ATBF and function

As mentioned above, adipose tissue expansion may result in adipocyte hypertrophy and hypoxia, which contribute to the adipose tissue dysfunction and a pro-inflammatory

state [77, 228]. However, as the main function of ATBF is to regulate metabolic homoeostasis, adipose tissue is highly vascularised [229]. ATBF is able to regulate to store and remove lipids when needed such as during stress, exercise and fasting [230]. Adipose tissue controls its metabolism in part by regulating its blood flow [99, 231]. This is because blood flow delivers key nutrients (e.g. oxygen and glucose) and hormones (e.g. insulin) and removes waste products for individual tissues [232]. ATBF is highly dependent on nutritional status, increasing significantly postprandially in healthy individuals [233-236].

However, ATBF is impaired in obese or IR individuals [122, 234, 235, 237, 238]. In adipose tissue, microvascular blood volume (MBV) and microvascular blood flow (MBF) have been shown to be impaired in IR mice [122]. Belcik, et al. performed abdominal adipose tissue perfusion imaging in obese IR (*db*/*db*) mice at 11-12, 14-16 weeks of age, and control lean mice. They found *Db*/*db* mice not only had higher fasting blood glucose and glucose area-under the curve (AUC), but also had lower adipose MBF and MBV (represents the numbers of capillaries open in adipose tissue). Also, the glucose AUC was positively correlated with adipose tissue MBF ($r = 0.81$) and MBV ($r = 0.66$) [122]. Recently, high-fat diet fed rats were demonstrated to have impaired capillarization and postprandial blood flow in adipose tissue and demonstrated that such events were associated with lower insulin sensitivity [238].

Adipose tissue MBF is impaired at baseline and postprandially in obese or IR status subjects [234, 235, 237]. For example, ATBF was impaired in the obese subjects, compared with the age-matched non-obese ($P < 0.05$) [237]. Frayn and colleagues have shown that the postprandial ATBF response is attenuated in obesity [234, 235, 239]. They also suggested that impaired regulation of postprandial ATBF is another facet of the insulin resistance syndrome [234]. Moreover, microvascular dysfunction is associated with decreased insulin sensitivity [240] and postprandial ATBF is significantly positively with insulin sensitivity [234, 241].

1.4.2 Regulators of ATBF

ATBF is very labile [242] and there are several factors that regulate ATBF (Table 1.1). After an overnight fast, the abdominal subcutaneous ATBF is typically about 3-5 mL

blood per 100 g adipose tissue per minute, while it is about 1.5 mL in skeletal muscle [243]. Interestingly, adipose tissue increases blood flow at night [244]. A 14 h -22 h fast does not change blood flow [245], but 72 h fasting improves the blood flow to adipose tissue to approximately 1.5 fold [246].

Moreover, ATBF increases up to four-fold in response to a meal in healthy people [233-235]. Also, ATBF reaches a peak at about 30 minutes following a mixed meal [235]. When glucose loading or a mixed meal was given to participants, the ATBF of healthy people was gradually increased several fold and then decreased to the baseline [100, 234, 235]. Introduction of N_G-monomethyl-l-arginine (L-NMMA), which is a blocker of nitric oxide (NO) synthesis, reduced fasting ATBF by 30 - 50%, suggesting that the endothelial NO system at least in part contributes to normal ATBF homeostasis [247, 248]. However, as mentioned above, this increase in ATBF induced by meals is impaired by obesity or IR [122, 234, 235, 237, 238].

Compared with skeletal muscle blood flow which can increase blood flow by 20-fold in response to exercise [242], ATBF increases up to around two-fold during exercise in healthy participants [249-252]. Interestingly, a small but significant increase in ATBF was observed during exercise in people with T2D [250]. The blood flow immediately dropped back to baseline levels after 60 minutes exercise and remained at this level for the remaining of the study [250]. However, ATBF in young, healthy subjects is much higher than that in people with T2D during and after exercise [249, 253].

Adrenaline is another important regulator of ATBF, as it exerts effects on both adipocytes and the vascular bed by acting through α1- and α2 – adrenoceptors, and β1- and β2-adrenoceptors (promoting vasoconstriction and inhibition of lipolysis) [254]. In humans, β-adrenergic stimulation significantly increases ATBF following β-adrenergic infusion or local delivery of β-adrenaline [255, 256]. However, previous reports have shown that α-adrenergic stimuli has an inhibitory effect on fasting ATBF [257, 258]. Ardilouze and colleagues illustrated that NO mainly regulates fasting ATBF and to some extent α-adrenergic system controls ATBF and β - adrenergic system principally regulates ATBF during postprandial status [247].

Table 1.1: Factors that regulate ATBF. ↑ Increase; ↓ Decrease.

Factors	Type of regulation	References
Fasting	↑	[242], [243], [244]
Meals	↑	[100], [231], [232], [233]
N_G-monomethyl-l-arginine	↓	[245], [246]
Obesity	↓	[233], [235]
Insulin resistance	↓	[122], [236]
Exercise	↑	[247], [248], [249], [250], [251]
α- adrenergic stimulation	↓	[255], [256]
β - adrenergic stimulation	↑	[253], [254]

1.4.3 Methods for measuring ATBF

There are several methods for determining ATBF described in previous studies. Most of these techniques are suitable for measuring subcutaneous adipose tissue perfusion, whereas visceral adipose tissue perfusion can only be measured by means of positron emission tomography (PET) [237].

PET is one of methods for measuring ATBF in humans [237, 251, 259]. In a PET scan, radioisotopic water [^{15}O]-H2O is used as a positron-emitting tracer to measure blood flow in adipose tissue [237, 251, 259]. Before the PET experiments, the antecubital vein is cannulated for tracer administration [251]. The fractional rate of tracer uptake is quantified by using graphical analysis of subcutaneous adipose tissue and visceral adipose tissue time-activity curves in 3D mode [259]. However, disadvantage of PET in a clinical research is the high cost [260].

Laser Doppler flowmetry (LDF), which uses laser light to monitor the movement of red blood cells, is a non-invasive tool to investigate blood flow changes in subcutaneous adipose tissue of people [261, 262]. The principle of LDF is that a fiber-optic probe is

attached to or inserted into the investigated tissue such as subcutaneous adipose tissue, and the Doppler shift of laser light occurs during light scattering by moving red blood cells. The amount and velocity of the blood flow in subcutaneous adipose tissue are measured by analysing the periodicity content of the backscattered light [261]. The LDF technique is easily performed and minimally invasive and has an excellent time resolution [261]. However, LDF has disadvantages as the signal is often collected superficially, so it can be difficult to determine the influence of skin blood flow on measurements [99].

Microdialysis using small molecules such as ethanol has been used to measure small changes of blood flow in adipose tissue [263-266]. Microdialysis probes are inserted percutaneously into the subcutaneous adipose tissue, and when ethanol is included in the perfusion solution, the ethanol clearance from the probe is a measure of tissue blood flow [263-265]. However, microdialysis is not able to measure rapid changes in blood flow, while limits the usefulness of this technique [266-268].

The ^{133}Xe-washout technique for determining ATBF has been the most widely applied since its introduction in 1966 [269]. The technique is based on the principle that the clearance of a substance injected into a tissue depends on blood flow through the tissue [270, 271]. Frayn and colleagues have developed and used this method to measure ATBF in people [229, 234, 235, 247, 272]. Using this technique, they have demonstrated that total ATBF increases: (i) post-prandially [235]; (ii) in response to an oral glucose challenge [247]; and (iii) during insulin infusion (euglycemic hyperinsulinemic clamp) [273]. Notably, since ^{133}Xe is highly lipid soluble, having a high distribution coefficient, it allows extended examination on the same area.

Recently, contrast-enhanced ultrasound (CEU) has been developed to measure ATBF in animals and humans [122, 274-276]. This was made possible in part with the development of the CEU technique for skeletal muscle [276-279]. Over the past 15 years the importance of microvascular blood flow in skeletal muscle for insulin action has been demonstrated by using CEU [296-302]. Briefly, a transducer interfaced with an ultrasound system is placed on the subcutaneous adipose tissue depot right of the umbilicus. Microbubbles (contrast agent) are continuously infused intravenously for imaging. Once the microbubble levels have reached steady-state (5 min) in the whole

body, a high energy destructive pulse of ultrasound is transmitted to destroy microbubbles within the volume of adipose tissue being imaged. Digital image analysis of the reflow of microbubbles into the tissues is performed off-line to calculate MBF rates [276]. The advantage of CEU over other techniques is the capacity to measure blood flow in the microvasculature (rather than bulk blood flow) and to isolate the microvascular responses − in particular, MBV, velocity and MBF [280, 281].

Indeed, the CEU technique has the capacity to isolate the measurement to the microcirculation and dissect different perfusion components – in particular, MBV (representing the number of capillaries being perfused), microvascular flow velocity (β – the filling rate of the capillaries being perfused) and MBF (which is the product of MBV and β) [280, 281]. This technique has recently been used to demonstrate that MBV in adipose tissue increases (microvascular recruitment) in response to an oral glucose tolerance test [274], and insulin infusion (euglycemic hyperinsulinemic clamp or intraperitoneal insulin injection) [116, 122, 274, 276].

1.5 Management of T2D

Lifestyle modifications and metformin monotherapy are recommended as the first steps to treat T2D [282, 283]. The major goal of this treatment is to control blood glucose levels and HbA1C within the normal range [282, 283]. However, most people with T2D will eventually require further treatment with more than one anti-diabetic drug to maintain their glycaemic goals, because T2D is a progressive condition [284]. Indeed, many people with T2D on monotherapy will not be able to maintain glycaemic control after just a few years and additional drugs will have to be introduced [285]. Moreover, combination of two or more pharmacological agents for managing T2D is often still unable to restore normoglycaemia over the long term [286]. In addition, the current treatment medication options often have negative side effects, such as body weight gain, hypoglycaemia and increased risk of cardiovascular diseases [286, 287]. Therefore, there is an urgent need to develop new treatment strategies to combat the increasing incidence of T2D.

1.5.1 Exercise and resistance training

Exercise is typically one of the first treatment strategies to combat obesity and T2D and to improve insulin sensitivity and reduce obesity-induced chronic inflammation [288, 289]. The American Diabetes Association (ADA) and the American College of Sports Medicine (ACSM) have exercise guidelines for the prevention and treatment of T2D based on multiple large randomized controlled trials [290, 291]. Also, the Australian National Heart Foundation (NHF) has suggested that all people with T2D should undertake 30 minutes or more of moderate exercise on most or all days of the week [292]. Regular chronic exercise has been illustrated to improve glucose tolerance, insulin sensitivity and lipid profiles [293-296]. Elevated HbA1c levels are predictive of vascular complications in T2D people, and regular exercise has been shown to reduce HbA1c levels, both alone and in conjunction with dietary intervention [289].

Moreover, regular long-term exercise has been shown to promote healthier skeletal muscle, liver, adipose tissue and pancreas function [289] (Figure 1.3). For example, regular exercise enhances skeletal muscle glucose uptake [297], and results in sustained improvements in insulin sensitivity and glucose disposal [298], and improves microvascular blood flow in skeletal muscle [299]. As with skeletal muscle, IR is also present in the liver in subjects with T2D, but exercise training intervention has been suggested to reduce hepatic IR, improve glucose uptake and insulin sensitivity [300, 301]. Also, exercise training provides numerous positive effects on adipose tissue, for example fat mass reduction, insulin sensitivity improvement, and reduced levels of inflammation [302, 303].

However, the relationship between exercise and inflammatory cytokine levels is complicated. During acute exercise, IL-6 is released from the contracting skeletal muscle cells into the blood [304]. As IL-6 has been considered a classic pro-inflammatory cytokine, the first possible explanation is that the IL-6 response was related to muscle damage. Also, IL-6 has been suggested to be an interleukin that works as both a pro-inflammatory cytokine and an anti- inflammatory cytokine [174, 175], so elevated IL-6 induces a subsequent increase in the production of IL-1 receptor antagonist (IL-1ra) and IL-10 by blood mononuclear cells, thus stimulating the occurrence of anti-inflammatory cytokines [305]. This is because IL-1ra inhibits IL-1β

signal transduction [305], and IL-10 is suggested to inhibit synthesis of pro-inflammatory cytokines such as TNF-a [175]. Indeed, an experimental study has confirmed that increased IL-6 acts to inhibit the production of TNF-α during physical exercise [306].

Regular exercise has been suggested to reduce pro-inflammatory markers such as TNF-α, IL-6, CRP and MCP-1. For example, a combined diet and exercise intervention suggested that TNF-α, IL-6, CRP and MCP-1 in adipose tissue of obese people significantly decreased after 15-week exercise training [307]. Interestingly, sVCAM-1 levels were significantly decreased after 15 weeks of low volume high intensity swim training [308]. However, other work has suggested that inflammation e.g. TNF-α, IL-1β, IL-6, CRP and MCP-1 does not change after exercise training [133, 309-312]. Exercise training had no effect on inflammation such as TNF-α and IL-6 in subjects with T2D exposed to a 12 week intervention period after a one year follow up [312].

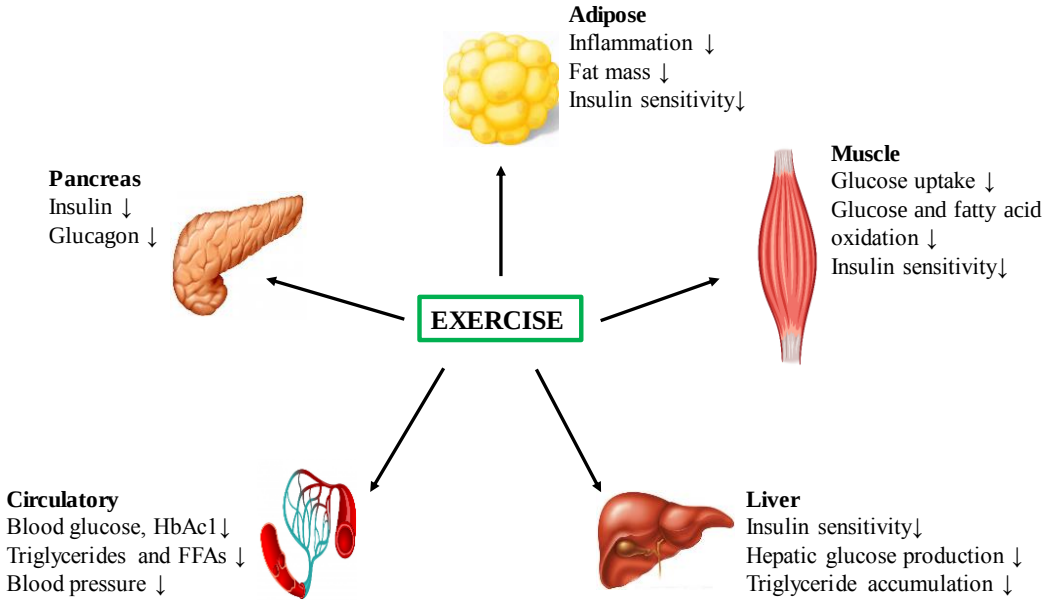

Figure 1.3: Benefits of exercise training on specific tissue in people with type 2 diabetes.

Recently, resistance training (RT) has gained considerable recognition as a viable exercise training option for subjects with T2D [313]. RT is an exercise that increases muscle strength through an external resistance or own body weight with the expectation of increases in strength, tone, mass, and/or endurance [314]. A RT program has also been recommended for people with T2D by the American Diabetes Association (ADA) and the American College of Sports Medicine (ACSM) [315, 316]. These two organizations recognize the positive effects of RT and recommend it no less than twice per week for people with T2D [317]. A randomized controlled trial has suggested that RT is able to improve glycaemic control in people with T2D, enhance glucose disposal, and even improve the lipid profile and reduce cardiovascular system disease risk for people with T2D [318, 319]. Also, RT is able to improve insulin sensitivity through enhancing the amount of GLUT4 protein, improving total muscle mass and increasing the number of insulin receptors on myocytes [320].

Moreover, RT has been found to increase skeletal muscle blood flow in humans [321-323]. Studies suggest that the ATBF increases in healthy humans during and after acute exercise [249, 251, 324, 325]. Also, acute exercise has been shown to improve ATBF in obese children [326]. There are very few studies that have investigated the effects of chronic exercise interventions on human ATBF. To date, most studies on human ATBF, such as those of Frayn and colleagues [99, 101, 272] have used ^{133}Xenon washout which measures the disappearance of the isotope injected into adipose tissue where faster disappearance reflects higher blood flow in adipose tissue. Using this technique it has been reported that ATBF is higher in trained *versus* sedentary healthy individuals [327, 328]. Sixteen weeks of endurance exercise training in young healthy lean men improves aerobic capacity by ~25%, but does not improve body composition (fat mass or lean muscle mass) or resting or epinephrine stimulated ATBF [329]. Similarly, 12 weeks of aerobic exercise training in healthy older women produced a significant increase in exercise capacity, but again, this improvement was not associated with changes in body composition or resting ATBF [330]. There have also been mixed findings regarding the impact of chronic exercise training (12-16 weeks) on ATBF in overweigh/obese individuals when assessed indirectly using microdialysis [331, 332]. Others have shown that RT intervention can reverse microvascular impairments in skeletal muscle and improve metabolic function of people with T2D [299]. However, the effect of RT on ATBF of people with T2D is still unknown.

1.6 Aims and hypotheses

Microvascular blood flow is important for delivery of key nutrients (e.g. oxygen, lipids and glucose) and hormones (e.g. insulin) to and removal of waste products from adipose tissue. Total ATBF increases after a meal, and this response is impaired in obesity and T2D. Microvascular blood flow is more important than total blood flow for nutrient exchange in many tissues, however it is not known whether microvascular blood flow in adipose tissue is altered by meals or T2D. Chronic exercise training improves microvascular blood flow in skeletal muscle of people with T2D. Whether adipose tissue microvascular responses are similarly improved following exercise training in people with T2D is unknown. The overarching goal of the current thesis is to characterise microvascular ATBF responses to a meal in healthy and T2D subjects, and

determine whether these responses are altered by chronic exercise training. Figure 1.4 describes the overarching current project's hypothesis and aims.

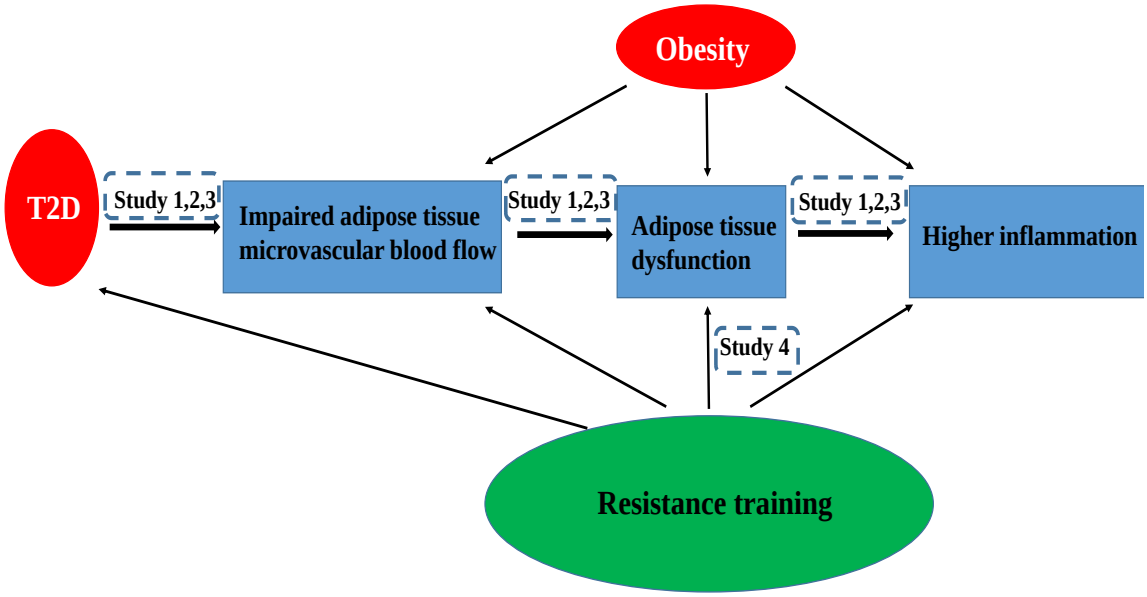

Figure 1.4: Summary of hypothesis and the aims of the current project.

Aims and hypotheses of project

Hypothesis 1:

Adipose tissue microvascular blood flow increases in the post-prandial state in healthy people.

Aim 1:

To characterise post-prandial adipose tissue microvascular blood flow responses in healthy subjects (Study 1, 2).

Hypothesis 2:

People with T2D have impaired post-prandial adipose tissue microvascular responses.

Aim 2:

To investigate post-prandial adipose tissue microvascular responses in people with T2D (Study 3).

Hypothesis 3:

Metabolic benefits of RT in people with T2D are linked to improvements in adipose tissue microvascular blood flow.

Aim 3:

To determine whether RT alters post-prandial adipose tissue microvascular responses in people with T2D (Study 4).

Chapter 2: General methods

This thesis includes four research projects involving humans. All studies were carried out in accordance with the Declaration of Helsinki as revised in 2008. All studies were approved by the University of Tasmania Human Ethics Committee (# H14086).

2.1 Recruitment

Participants were recruited through community advertisement such as radio, flyers, website and patients from previous studies that gave consent to be contacted for future studies. An initial screening was conducted through a health history questionnaire (over the phone or via email) to rule of any exclusion criteria prior to their first visit. Participants who met the inclusion criteria and exclusion criteria were invited to come into the clinic for a screening visit. For T2D people, they were all diagnosed by another doctor (general practitioner or endocrinologist) based on their blood glucose levels (non-fasting blood glucose >11.0mmol/L, fasting blood glucose >7.0mmol/L, or 2 hours post oral 75g glucose tolerance test >11.0).

2.2 Participation criteria

Participants who satisfied the inclusion and exclusion criteria were invited to come in to clinic centre, Menzies Institute for Medical Research, University of Tasmania for a screening visit.

Inclusion criteria:

1. Aged between 18 - 60 years old.
2. Healthy or have clinically diagnosed type 2 diabetes.
3. Normal weight or overweight (BMI ranging from 18- 35 kg/m^2).
4. Have normal or controlled blood pressure (seated brachial blood pressure < 140/90 mmHg).

Exclusion criteria:

1. Age <18 years or >60 years.

2. Morbidly obese with a BMI ≥ 35 kg/m^2.
3. Cardiac disease with symptoms at rest or inducible with exercise.
4. History of myocardial infarction or stroke.
5. Exercise capacity limited by a factor other than claudication, for example: coronary artery disease (angina pectoris), pulmonary disease, arthritis or other musculoskeletal complication.
6. Critical limb ischemia including peripheral artery disease or previous revascularisation or other surgical treatment for peripheral artery disease.
7. History of malignancy within past 5 years (except for non-melanoma skin cancers).
8. Identification of any medical condition requiring immediate therapeutic intervention.
9. Uncontrolled hypertension (resting brachial blood pressure ≥140/90 mmHg).
10. Current smoker.
11. History of severe liver disease.
12. History of drug or alcohol abuse.
13. Elective major surgery during the course of the study.
14. Pregnancy/lactation.
15. For T2D participants in the RT program - participation or intention to participate in a structured and/or supervised physical activity program during the study period.
16. Participation or intention to participate in another clinical research study during the study period.

2.3 Study protocol

2.3.1 Screening visit

Participants were invited to come into the Menzies Institute for Medical Research clinical centre. After signing the informed consent form, their eligibility was confirmed by using medical questionnaires, measuring their blood pressure (brachial blood pressure was recorded using OMREN HEM 907 device in duplicates one minute apart), height and weight. Participants who qualified were invited to complete a physical activity questionnaire (IPAQ) to monitor physical activity before each clinic visit. Patients who were deemed eligible made an appointment for further testing.

2.3.2 Clinic visit

Participants fasted overnight prior to each clinic visit (no food for 12 hours), they refrained from vigorous exercise and alcohol for 48 hours prior to testing, and refrained from caffeine on the morning of the study. T2D participants stopped taking oral medications e.g. metformin, for 48 hours prior to testing. All participants rested in an adjustable bed in a semi-recumbent position. Participants resting for 30 mins prior to testing and throughout the clinical testing. A small polyethylene catheter was placed into an antecubital vein of one arm for blood sampling and for infusing contrast agent (Definity). Further details about the clinical testing are outlined below.

2.3.3 Body composition

Body composition was determined for all participants by dual-energy X-ray absorptiometry (DEXA, Hologic Delphi densitometer, Hologic, Waltham, USA). Briefly, the scanner incorporates a constant potential X-ray source at 76 kV and a K-edge filter (cerium) to achieve a congruent beam of stable dual-energy radiation, 38 keV and 70 keV. The beam undergoes attenuation by the tissues as the X-ray beam passes through the participant. An experienced radiology technologist performed all DEXA examinations and the total body scanning time was about 6 minutes. Total and trunk body fat percentage were calculated using Hologic Apex System software version 4.02 [333].

2.3.4 Oral glucose challenge and mixed meal challenge

Participants undertook a 2-hour oral glucose challenge (OGC, 50g) or a mixed meal challenge (MMC, 1241 kJ; 4.8g fat, 41g carbohydrate, 21.7g protein, and 25.1g sugars) to assess their glucose tolerance, microvascular responses and insulin sensitivity. A mixed meal was chosen to assess the physiological changes that occur in response to a typical meal. A liquid meal was administered to shorten the amount of time needed for digestion and absorption. The subjects drank the glucose or the meal within five minutes. Before the OGC or the MMC, plasma and serum samples were collected and later sent to Royal Hobart Hospital Pathology for the measurement of fasting glucose, lipids, and HbA1c. After the ingestion of the drink, blood samples were collected at 15, 30, 60, 90, and 120 minutes for the measurement of glucose and insulin concentrations. Plasma

glucose was measured by using an YSI analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin was assayed by using ELISA (Mercodia, Sweden). The blood collection tubes were immediately immersed in ice and centrifuged at 2400 *g* for 10 min, and samples were frozen and stored at -80°C until analysis.

2.3.5 Adipose tissue microvascular responses by real-time contrast enhanced ultrasound (CEU)

Central (truncal) subcutaneous adipose tissue microvascular blood flow was assessed by real-time CEU (Figure 2.1). The techniques measures blood vessels that are < 40 μm in diameter. However, the majority (>90%) of the vessels in this size range are microvascular (5-10 μm diameter). A linear array transducer (L9-3) interfaced with an ultrasound system (iU22, Philips Medical Systems, Australia) was placed horizontally over the abdomen (immediately right of the umbilicus) and the beam focused on the subcutaneous adipose tissue depot. Microbubbles (Definiyt, Lantheus Medical Imaging, Melbourne, Australia) were diluted (1.5ml added to 30ml saline) and continuously infused intravenously at 2.0-2.6 ml/min (equating to 0.03 ml/min/kg body weight) for adipose tissue imaging. Once the systemic microbubble concentration reached steady-state (5 min), a high energy destructive pulse of ultrasound was transmitted to instantaneously destroy microbubbles within the volume of adipose tissue being imaged. The reflow dynamics of microbubbles into adipose tissue microvasculature was assessed in real-time at baseline and then repeated 1hr following an OGC or a MMC.

Ultrasound settings including gain settings were optimized in humans (after initial *in vitro* experiments) to ensure 1) a high signal-to-noise ratio and 2) to confirm imaging within the linear portion of the microbubble concentration *versus* acoustic intensity curve. This is achieved by quantifying tissue and microbubble acoustic intensities under a variety of settings and microbubbles infusion rates. Gain settings (90%), mechanical index (0.11 for continuous and 1.30 for flash), compression (C=30), depth and focus were identical between healthy controls and those with T2D.

Figure 2.1: Ultrasound imaging on subcutaneous central adipose tissue (Left) and example image containing contrast microbubbles (Right).

2.3.6 Image analysis

Digital image analysis was performed off-line using Qlab (Philips Medical Systems, Australia). Images were background subtracted (0.5 sec frames) as published previously to eliminate signal from larger blood vessels and tissue *per se* [334]. Analysis of the data was performed identically for baseline and 1h after MMC or OGC. Briefly, refilling measures were taken in triplicate and then averaged, with the signal from the larger vessels and background subtracted (0.5 second). The microvascular refilling curve was created by plotting time (seconds) verses acoustic intensity (AI), giving an exponential rise to maximum plot (Figure 2.1). Microvascular volume (A) and microvascular filling rate (β) where determined from the graph via SigmaPlot graphing software (Systat Software Inc., CA, USA) with the equation:

$$Y = A(1-e^{-\beta(x(t)-bkg(t))})$$

Where:

X = Time (secs)

Y = Acoustic intensity

A = Acoustic intensity at the plateau position (an indicator of microvascular volume)

β = Rate constant which provides a measure of the microvascular filling rate

bkg = time of background image

Microvascular blood flow is calculated by multiplying A and β values.

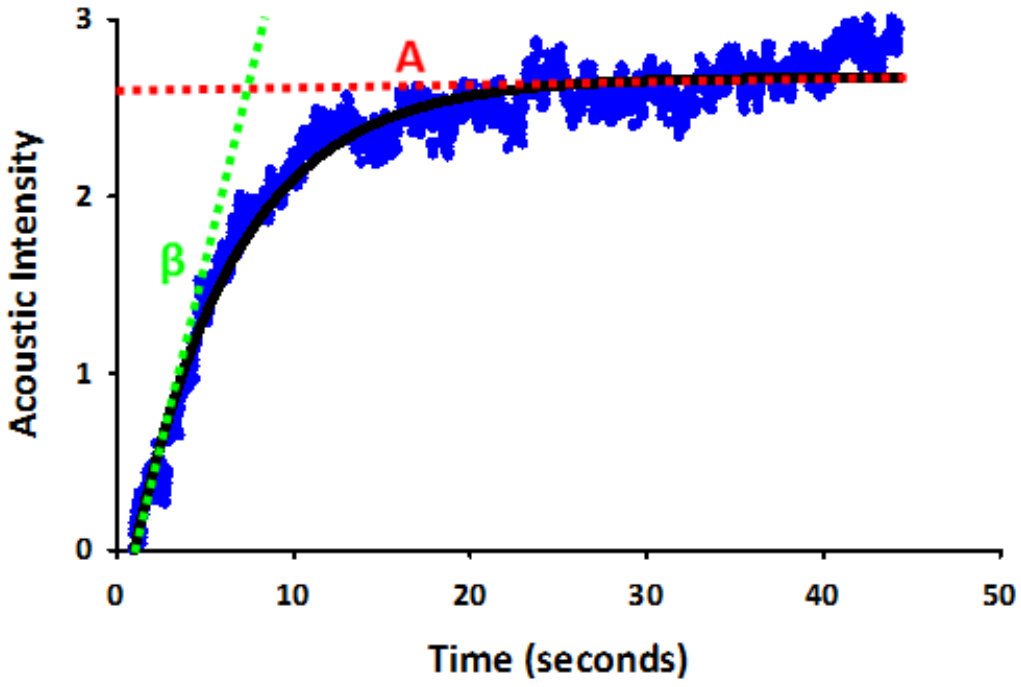

Figure 2.2: Microvascular blood volume and filling rate determination. Plot was constructed after high energy ultrasound pules destroyed microbubbles in imaged region at t=0. Microvascular blood volume is indicated by the plateau position (A), and the rate of microvascular refilling is indicated but the tangent to the AI rise (β). Microvascular blood flow (also known as perfusion) is calculation by A × β.

2.3.7 Inflammatory cytokines

Plasma concentrations of TNF-α, IL-1β, IL-6, CRP, MCP-1 and sVCAM-1 were measured using commercially available enzyme-linked immunosorbent assay (ELISA) (ELISAKIT, Australia). The protocol was performed as per manufacturer's instructions.

2.3.8 Free fatty acids (FFAs)

FFAs levels were determined by using an enzymatic assay kit (Wako Pure Chemical Industries, Osaka, Japan). The protocol was performed as per manufacturer's instructions.

2.3.9 Resistance training

Some of the T2D participants in the study completed six weeks of a resistance training (RT) program. Details of the RT program are described in chapter 6. In summary, before and after the RT, participants fasted overnight and came to the clinic to conduct the same metabolic and adipose tissue microvascular blood flow testing as described above.

2.3.10 Summary of the testing protocol per visit

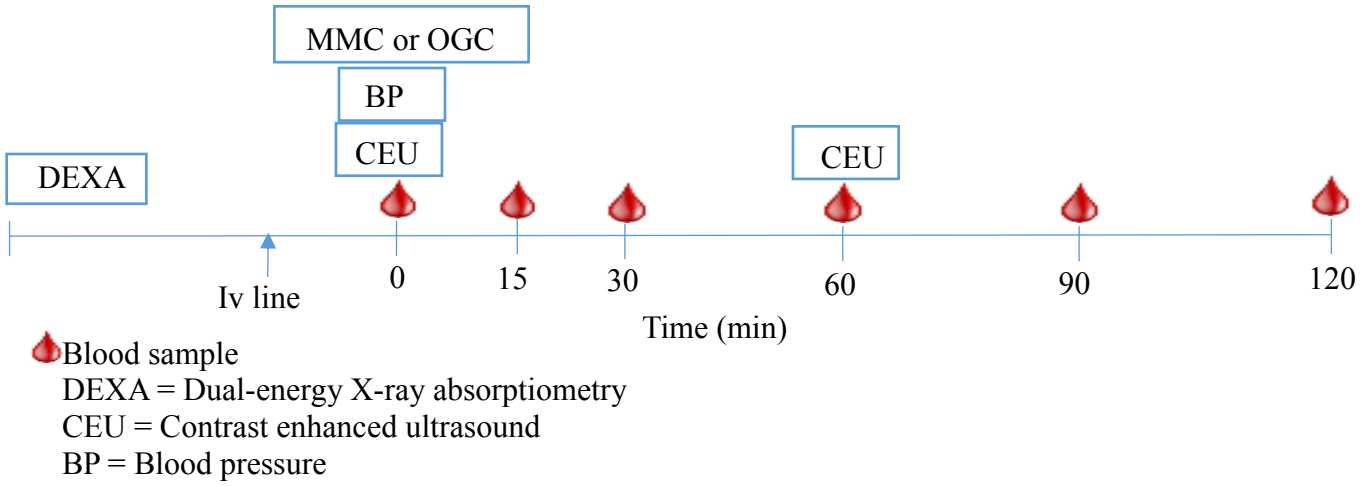

Figure 2.3: Participants fasted overnight prior to their clinic visit. The metabolic and adipose tissue microvascular blood flow testing took about 3 hours to complete.

2.4 Statistical analysis

Data are presented as the means ± SEM and statistics were performed using SigmaPlot (Systat Software, San Jose, CA, USA). One way or Two-way repeated measures ANOVA with Student-Newman-Keuls post hoc test was used to compare treatment

groups over the time course of experiment where appropriate. Students paired or un-paired t-test was used to make comparisons between single point measurements where appropriate. When data were not normally distributed the Signed Rank Test or Wilcoxon rank sum test was performed where appropriate. For categorical variables a Fisher's exact test was performed. Pearson's bivariate correlations were used to evaluate associations. Spearman correlations were used to evaluate associations when data were not normally distributed. A value of $P < 0.05$ was considered as statistically significant.

Chapter 3: Postprandial adipose tissue microvascular blood flow in healthy subjects

3.1 Introduction

Adipose tissue has long been known for its capacity to store triglycerides (TG), dispose of glucose after a meal and release FFAs [101, 254]. Blood flow in adipose tissue is important for delivery of nutrients (e.g. lipids, glucose), hormones (e.g. insulin) and oxygen to the adipocyte, and allows release of hormones and nutrients from the adipocyte to the general circulation [232]. To date, most studies on human ATBF, such as those of Frayn and colleagues [99, 101, 272] have used ^{133}Xenon washout which measures the disappearance of the isotope injected into adipose tissue where faster disappearance reflects higher blood flow in adipose tissue. Using this technique, they demonstrated that blood flow to adipose tissue increases: (i) post-prandially [235]; (ii) in response to an oral glucose challenge [247]; and (iii) during insulin infusion (euglycemic hyperinsulinemic clamp) [273]. However, given that capillaries regulate nutrient exchange, measuring vascular responses in adipose tissue at the microvascular level reveals more information about mechanisms of nutrient exchange. What happens at the microvascular level in adipose tissue after the ingestion of a meal has never been previously reported.

Nutrient exchange at the microvascular level can occur via an increase in the number of microvessels (capillaries) receiving blood flow to augment the endothelial surface area, or via an increase in the rate across the microvascular bed which can increase nutrient supply. The CEU technique has the capacity to isolate the measurement to the microcirculation and dissect different perfusion components – in particular, microvascular blood volume (MBV – the number of capillaries being perfused), microvascular flow velocity (β – the filling rate of the capillaries being perfused) and microvascular blood flow (MBF – which is the product of MBV and β) [280, 281]. Whether adipose tissue MBV, β and MBF are increased in response in the post-prandial state has not been previously reported.

The aim of this chapter was to determine whether adipose tissue microvascular blood volume and blood flow increases in the post-prandial state in healthy people, using the CEU technique.

3.2 Research design and methods

3.2.1 Screening visit

Healthy people were recruited from the community (see Table 3.1). For the screening visit, participants were invited to come into the Menzies Institute for Medical Research clinical centre to confirm their eligibility by using medical questionnaires. Subjects were excluded from the study if they had any of the following characteristics: a history of smoking; current pregnancy; cardiac disease; history of severe liver disease; history of drug or alcohol abuse; cancer in the last 5 years, elective major surgery during the course of the study or taking any medication known to affect glucose metabolism. All participants gave written informed consent.

3.2.2 Clinic visit

After the screening visit, 15 healthy controls (6F/9M) were eligible and were invited back to the Menzies Institute for Medical Research after an overnight fast. Participants refrained from exercise and alcohol 48hr prior to testing. Caffeine was omitted on the day of testing. All participants underwent the following testing.

3.2.3 Mixed meal challenge (MMC)

Mixed meal challenge has been described in Chapter 2.3.4. Participants rested for approximately 30 min before undergoing a mixed meal challenge (MMC). Subjects were then given a liquid MMC (1241 kJ; 4.8g fat, 41.0g carbohydrate, 21.7g protein, and 25.1g sugars – Table 3.2). A liquid meal was administered to shorten the amount of time needed for digestion and absorption. The subjects drank the meal within 5 min.

The degree of insulin sensitivity was assessed using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR)[335] and the Quantitative Insulin Sensitivity Check (QUICKI)[336] using the following equations:

HOMA-IR = fasting insulin x fasting glucose/405

QUICKI = 1/(log(fasting insulin)+log(fasting glucose)

Fasting insulin is expressed as μU/ml and fasting glucose is expressed as mg/dL. Elevated levels of HOMA-IR and low levels of QUICKI indicate a higher degree of insulin resistance.

3.2.4 Body composition

All participants underwent a whole body dual energy X-ray absorptiometry (DEXA) scan to assess body composition, in particular the amount of total body fat and trunk fat as described in Chapter 2.3.3

3.2.5 Real-time contrast enhanced ultrasound (CEU)

Central (truncal) adipose tissue microvascular blood flow was assessed by CEU as described in Chapter 2.3.5. Adipose tissue microvascular responses were measured at baseline and then repeated 1hr following the MMC. CEU was measured 1 hour postprandially as insulin excursions peak at approximately 1 hour following a meal.

3.2.6 Image analysis

Digital image analysis was performed off-line using Qlab (Philips Medical Systems, Australia) as described in Chapter 2.3.6. MBV, β and MBF were calculated based on curve fitting described in Chapter 2.3.6.

3.2.7 Statistical analysis

Data are presented as the means ± SEM and statistics were performed using SigmaPlot (Systat Software, San Jose, CA, USA). One-way repeated measures ANOVA with Student-Newman-Keuls *post hoc* test was used to determine the time course effect of the MMC. A Student's paired t-test was used to determine if there were differences between two time points. Pearson's bivariate correlations were used to evaluate associations. Spearman correlations were used to evaluate associations when data were not normally distributed. A value of $p < 0.05$ was considered as statistically significant.

3.3 Results

3.3.1 Characteristics of participants

The participant characteristics are presented in Table 3.1. Their anthropometrics, clinical chemistries and blood pressure were within a normal range. However, BMI and body composition (total fat content) indicate that the participants ranged from normal to obese.

3.3.2 Glucose and insulin responses to mixed meal challenge

Figure 3.1 shows the glucose and insulin responses to the MMC. Following the MMC, blood glucose rose modestly and reached a peak at 30 min (Figure 3.1A). Blood glucose gradually declined over time but remained significantly higher than baseline at 2hrs ($p < 0.001$). Insulin excursion had a similar time course and remained significantly higher than baseline during the 2 hrs post ingestion (Figure 3.1B) ($p < 0.001$).

3.3.3 Adipose tissue microvascular responses to mixed meal challenge (MMC)

Figure 3.2 shows examples of adipose tissue CEU images from a healthy participant showing intensity of microbubbles before and 1hr-post MMC (Figures 3.2A and B, respectively). Their respective curve fits are shown in Figures 2C and D. The region of interest was not identical and not exactly the same size between people. However, the same region of interest was used with repeated measures within the same person.

Figure 3.3 shows the average microvascular responses in all participants. MBV was significantly elevated 1-hr following the MMC when compare to baseline ($p = 0.008$) (Figure 3.3A). β was not altered by the MMC (Figure 3.3B). MBF was higher compared to baseline but was borderline significant ($p = 0.088$) (Figure 3.3C).

3.3.4 Correlates to adipose tissue microvascular responses

Correlations were conducted to determine associations with adipose tissue microvascular responses (MBV and MBF) for all healthy subjects (Table 3.3). Figure 3.4 shows the plots of these significant correlations. Baseline MBV was negatively associated with fasting insulin, but positively associated with QUICKI (a surrogate marker of insulin sensitivity). MBV following the MMC was positively associated with diastolic blood pressure (DBP). Also, baseline MBF was negatively associated with total body fat (%) and truncal fat (%), and post meal MBF was negatively associated with fasting TG levels.

Table 3.1: Characteristics of participants.

Characteristics	Mean ± SEM	Range
n	15	-
Age (years)	46 ± 2	22 - 57
Sex	6F/9M	-
Height (cm)	170.8 ± 2.8	152.0 -188.0
Weight (kg)	76.4 ± 2.5	58.4 - 93.0
BMI (kg/m2)	26.2 ± 0.8	21.7- 33.3
Body Fat		
Total fat (%)	28.1 ± 2.1	16.6 - 47.0
Trunk fat (%)	27.8 ± 2.1	16.7 - 46.7
Fasting glucose (mmol/L)	4.63 ± 0.18	2.67 -5.46
Fasting insulin (pmol/L)	42.6 ± 3.5	24.1- 80.1
HbA1c		
%	5.33 ± 0.06	4.80 -5.90
mmol/mol	34.7 ± 0.7	29.0 -41.0
Insulin Sensitivity Indices		
HOMA-IR	1.27 ± 0.12	0.61 -2.51
QUICKI	0.37 ± 0.01	0.33 - 0.42
Blood Pressure		
SBP (mmHg)	123 ± 2	107 - 139
DBP (mmHg)	78 ± 2	65 - 89
Lipids		
Cholesterol (mmol/L)	4.95 ± 0.23	3.30 - 6.80
Triglyceride (mmol/L)	0.86 ± 0.07	0.38 - 1.51
HDL (mmol/L)	1.49 ± 0.09	0.90 - 2.30
LDL (mmol/L)	3.06 ± 0.21	1.50 - 4.50
FFA (mmol/L)	0.40 ± 0.05	0.09 - 0.71

Data expressed as mean ± SEM. None of the subjects was on medication to treat obesity or lower lipids.

Table 3.2: Macronutrient composition of the mixed meal challenge (MMC).

	MMC
Energy (kJ)	1241
Protein (g)	21.7
Fat (g)	4.8
Carbohydrate (g)	41.0
Sugars (g)	25.1

Table 3.3: Correlates of adipose tissue MBV and MBF response to MMC

Characteristics	Baseline MBV		MMC MBV		Baseline MBF		MMC MBF	
	r	*p*	r	*p*	r	*p*	r	*p*
Body fat								
Total fat (%)	-0.348	0.204	0.124	0.660	**-0.713**	**0.002**	-0.154	0.585
Trunk fat (%)	-0.369	0.176	0.049	0.863	**-0.654**	**0.008**	-0.191	0.496
Metabolism								
Fasting glucose (mmol/L)	-0.211	0.441	-0.264	0.332	0.154	0.575	-0.446	0.092
Glucose AUC (mmol/L.120min)	-0.007	0.980	-0.118	0.676	0.154	0.575	-0.385	0.156
Fasting insulin (pmol/L)	**-0.540**	**0.038**	-0.365	0.181	-0.507	0.052	-0.197	0.482
Insulin AUC (mmol/L.120min)	0.018	0.944	-0.100	0.714	0.136	0.620	0.093	0.734
HbA1c (%)	0.078	0.783	-0.061	0.828	0.178	0.514	-0.199	0.477
QUICKI	**0.622**	**0.013**	0.400	0.140	0.429	0.107	0.154	0.584
Fasting serum triglyceride (mmol/L)	-0.311	0.259	-0.374	0.170	-0.302	0.263	**-0.626**	**0.013**
Fasting plasma FFA (mmol/L)	0.282	0.309	0.057	0.839	0.129	0.639	-0.304	0.270
Blood pressure								
SBP (mmHg)	0.146	0.604	0.288	0.299	0.189	0.489	0.227	0.415
DBP (mmHg)	0.385	0.156	**0.703**	**0.003**	0.271	0.319	0.261	0.069

Pearson's correlation was used between normally distributed variables. Spearman correlation was used if any of the variables were not normally distributed. Bold indicate significant correlations. MBV: Microvascular blood volume; MBF: microvascular blood flow; MMC: mixed meal challenge.

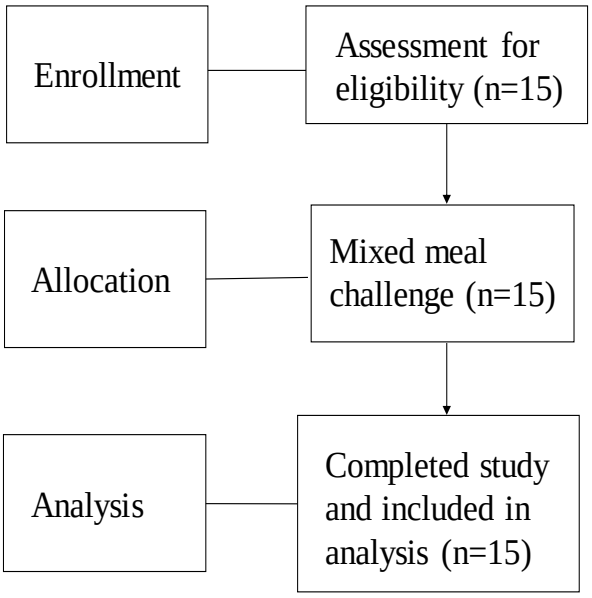

Figure 3.1: Flowchart of the number of participants screened, excluded, studied, and analysed.

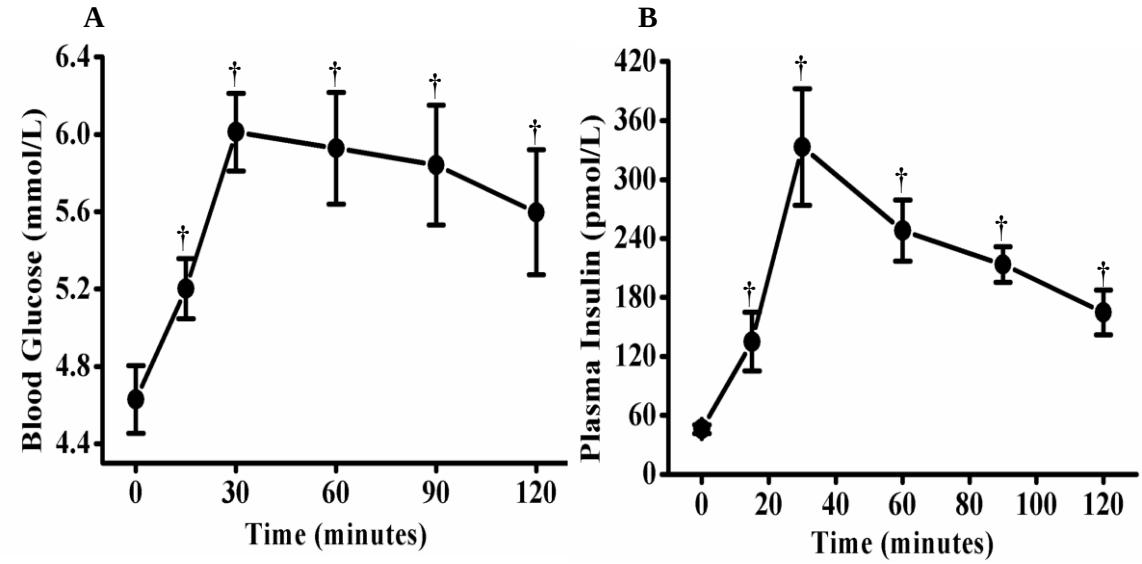

Figure 3.2: Blood glucose (A) and plasma insulin (B) timelines in response to the mixed meal challenge. Data are means ± SEM. Repeated-measures one-way ANOVA was used to determine if there were differences over the time course of the experiment. † $P < 0.01$ vs. baseline.

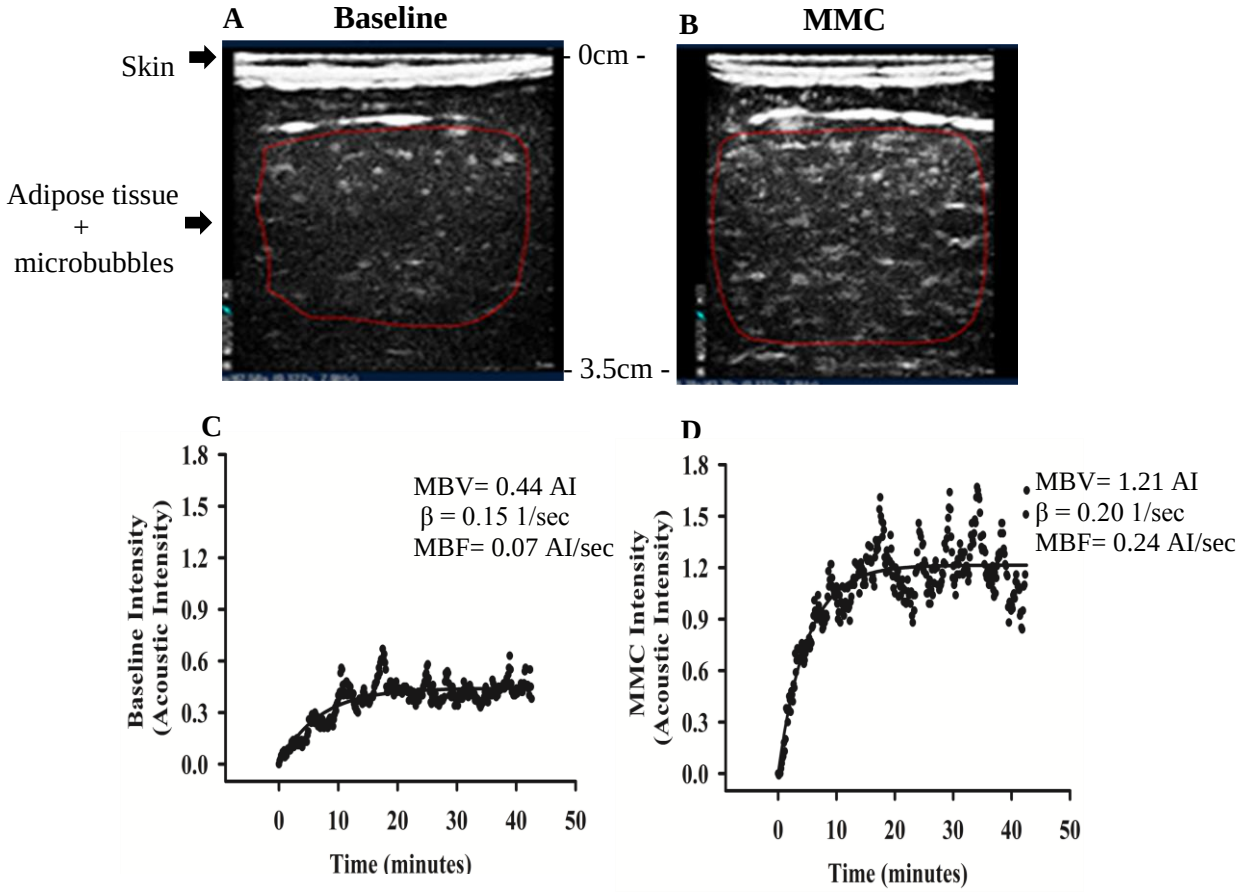

Figure 3.3: Examples of adipose tissue CEU images showing intensity of microbubbles before (A) and after (B) a 1hr mixed meal challenge (MMC) in a healthy individual. Red line indicates the region of interest for analysis. Corresponding curve fits after a destructive pulse of ultrasound before (C) and after (D) a 1hr MMC.

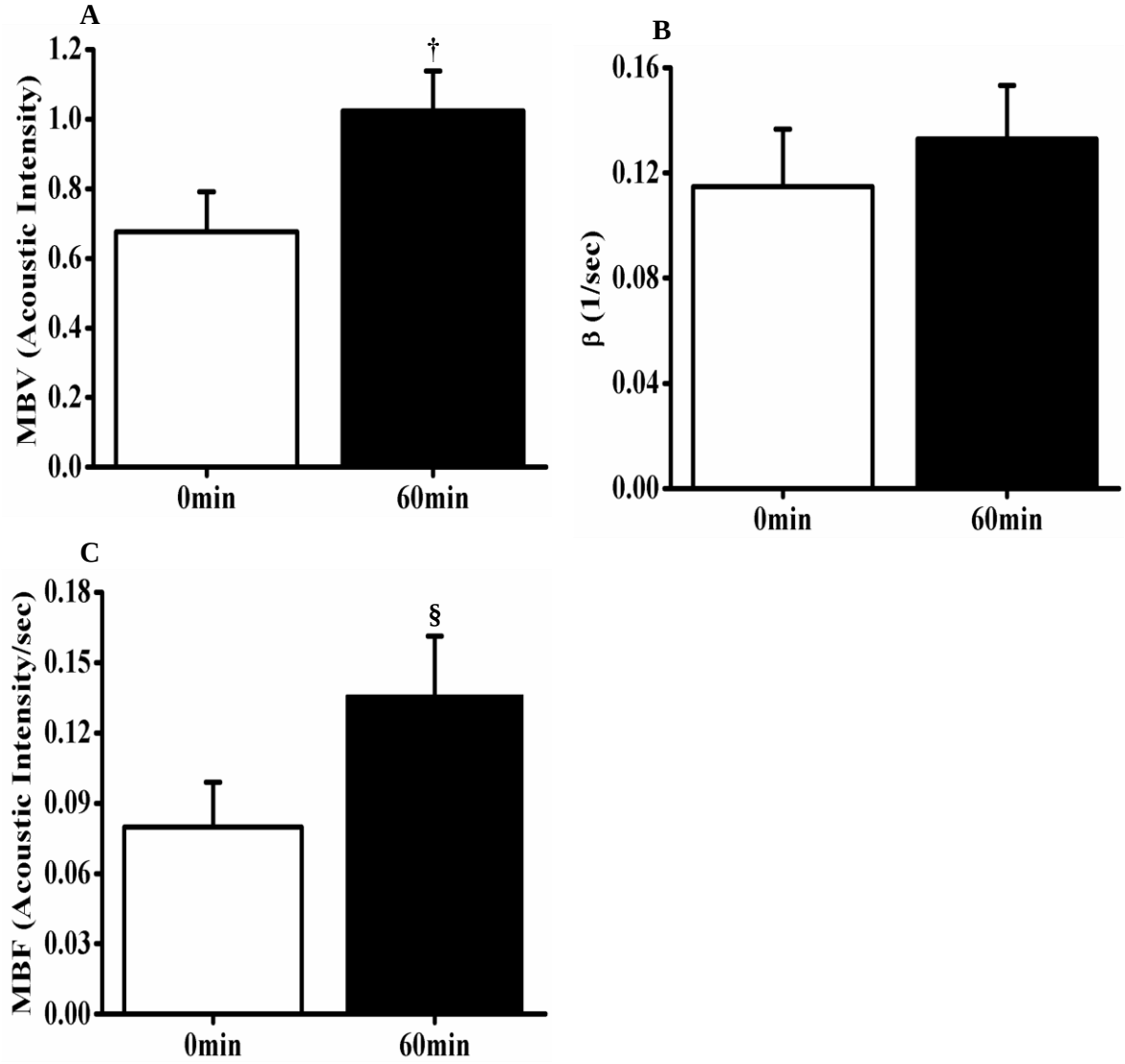

Figure 3.4: Adipose tissue microvascular responses the MMC in healthy people. MBV (A), β (B) and MBF (C) at time 0min (baseline) and 60min after MMC. Data are means ± SEM. A Student's paired t-test was used to determine if there were differences between the two time points. $^{\dagger}P < 0.01$ vs. baseline MMC; $^{\S}P = 0.088$ vs. baseline MMC. MBV: Microvascular blood volume; MBF: microvascular blood flow; MMC: mixed meal challenge.

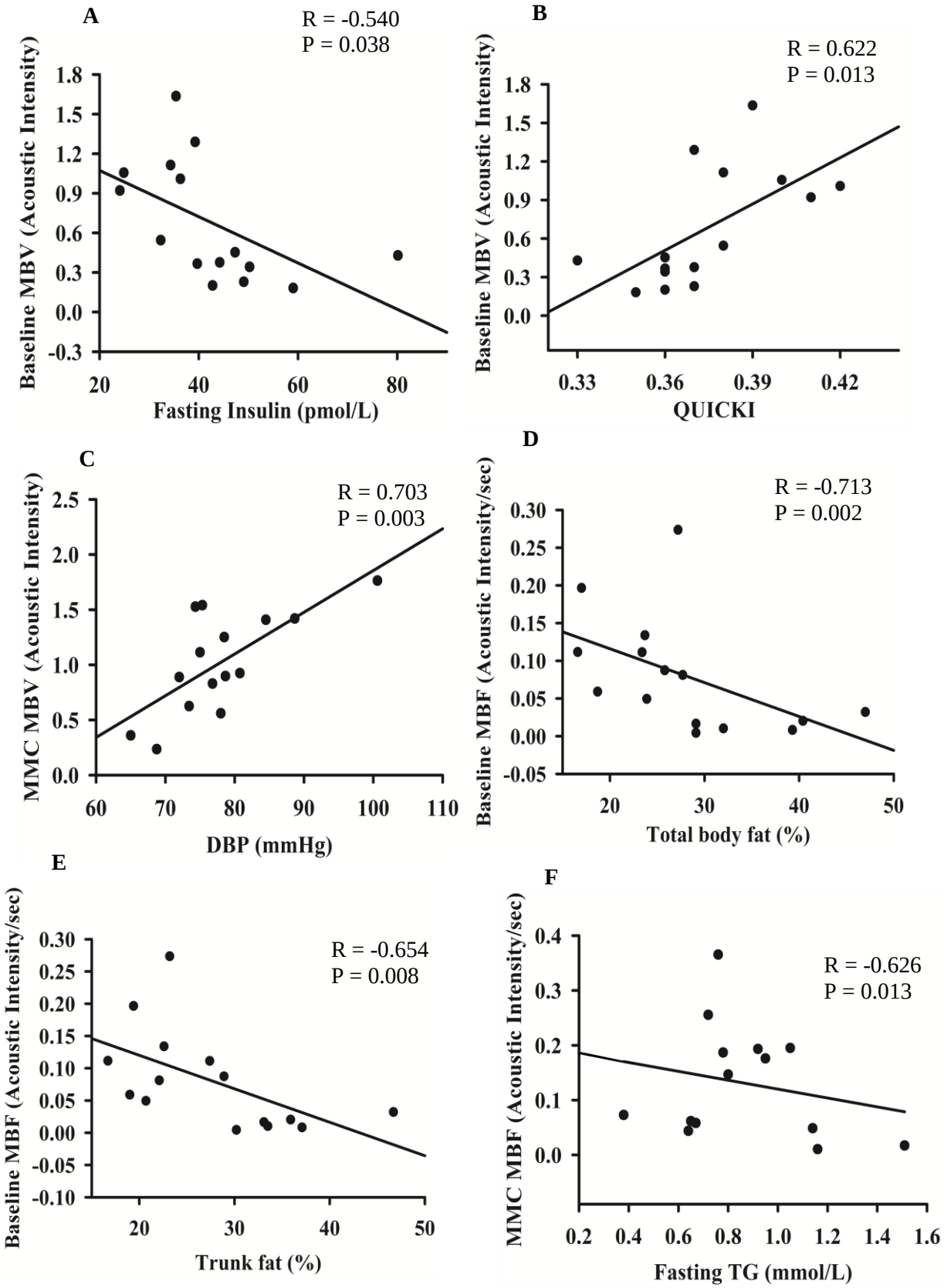

Figure 3.5: Statistically significant correlations for baseline MBV and fasting insulin (A), baseline MBV and QUICKI (B), MMC MBV and DBP (C), baseline MBF and total body fat (D),baseline MBF and trunk fat (E) and MMC MBF and fasting TG (F).

MBV: Microvascular blood volume; MBF: microvascular blood flow; MMC: mixed meal challenge.

3.4 Discussion

This is the first study to explore postprandial adipose tissue microvascular responses in healthy people. The key findings of this study were that adipose tissue MBV and MBF responses were significantly elevated following the MMC. This microvascular action may improve delivery of hormones (e.g. insulin) and nutrients (e.g. glucose and lipids) from the MMC to adipose tissue for storage.

Insulin increases MBF and recruits capillaries in skeletal muscle [277, 337-341]. Importantly, the MMC also markedly increases muscle MBV [342], thereby expanding the endothelial surface for nutrient exchange, and this vascular process is an important physiological response to facilitate nutrient/hormone delivery to skeletal muscle [340, 343]. Gersh and colleagues have suggested that adipose tissue possesses the ability to recruit capillaries to the same extend as that found in skeletal muscle [344], however these studies have not been previously conducted. The current study confirms that the MMC increases MBF and recruits capillaries (MBV) in adipose tissue in healthy subjects (Figure 3.2 and 3.3). Therefore increases in MBV and/or MBF may facilitate uptake of nutrients from the meal into adipose tissue.

The participants in the current study were all non-diabetic individuals (based on their fasting clinical chemistries). However, there was a large range in their BMI and their amount of body fat, spanning from healthy-to-overweight-to-obese. During obesity, adipose tissue expands via hypertrophy (increased adipocyte size) or via hyperplasia (increased adipocyte number), with the former being metabolically detrimental [106]. The current study demonstrated that the degree of obesity is a strong correlate of adipose tissue MBF (Figure 3.4). This is not a surprising finding given that others have demonstrated a similar relationship between bulk ATBF and obesity [235, 272, 345] and it is well established that with adipocyte hypertrophy, as occurs in obesity, there is a reduction in capillary density [346]. However, this is the first study to demonstrate this negative association between ATBF and adiposity at the microvascular level in humans.

It is intriguing that the relationship with MBF and adiposity was stronger than MBV considering the reduction in capillary density might be expected to reduce MBV. It was

anticipated that the degree of obesity would be a stronger predictor of MBV and it was surprising to find that their baseline MBV did not correlate with body fat. Histology was not conducted to assess capillary density and as such can only speculate that the capillary density was similar between lean and obese individuals, or that those with obesity had a greater number of capillaries perfused at rest. However, this study has demonstrated that those individuals with a greater amount of total body fat or trunk fat had a lower baseline adipose tissue MBF.

Using ^{133}Xe, ATBF increases (i) post-prandially [235]; (ii) in response to an oral glucose challenge [247]; and (iii) during insulin infusion (euglycemic hyperinsulinemic clamp) [273] and these vascular responses are impaired in subjects with IR [100, 234, 275] . It is not surprising that in the current project there is a positive relationship between the degree of IR (based on fasting insulin levels and QUICKI) and MBV. However, this is the first study in humans to demonstrate this relationship at the microvascular level in humans. Given that a positive association was observed between diastolic blood pressure and post-prandial MBV, one possibility is that higher blood pressure increases the number of capillaries open during the MMC. However the potential mechanism behind the positive association between MBV and blood pressure needs to be further investigated.

In summary, the current chapter has demonstrated that 1) adipose tissue microvascular responses are augmented in the post-prandial state in healthy people and 2) this microvascular effect is lower in people with obesity and with early signs of IR.

Chapter 4: Comparison between oral glucose challenge and mixed meal challenge on adipose tissue microvascular blood flow in healthy subjects

4.1 Introduction

Chapter 3 demonstrated that the MMC stimulates adipose tissue MBV and MBF in healthy people. This effect was blunted in healthy people who had a higher amount of body fat (in particular trunk fat). This microvascular action may regulate delivery of nutrients from the MMC (e.g. lipids and glucose) to adipose tissue for storage. The effects seen in adipose tissue in Chapter 3 were similar to the responses seen in skeletal muscle [338]. MBF and MBV in skeletal muscle are elicited by a meal or insulin infusion and that this response is impaired during IR such as obesity [342, 347].

It is plausible that the mechanism of this impairment is the same in the two tissues (i.e. skeletal muscle and adipose tissue) because they share a common insulin signalling pathway linking meal ingestion and vasodilation of the microvasculature [348]. It has been established that the mechanism in skeletal muscle is via insulin causing NO release from endothelium via endothelial insulin receptor, stimulation of Akt, and activation of eNOS [349, 350]. Mechanisms of vasodilation in adipose are less well-defined, but NO blockade similarly impairs the ATBF response to oral glucose loading [247], and others have shown that ATBF increases in response to insulin infusion [273, 276]. However, the increase in ATBF in response to insulin infusion is lower than that of oral glucose loading, suggesting additional vasodilatory signals may be involved [273]. Notably, β adrenergic receptor antagonism partially blocks the ATBF response to oral glucose loading, suggesting possible sympathetic nervous involvement in the vasodilation [247].

An additional intriguing observation that has recently been reported is that in skeletal muscle MBV increases in response to a MMC but is markedly impaired in response to an oral glucose challenge despite a similar level of hyperinsulinemia [351]. However, a direct comparison of adipose tissue microvascular responses to an MMC and an oral glucose challenge has not been made to date.

Therefore, the aim of this chapter is to compare the adipose tissue microvascular responses to an OGC and a MMC, while matching plasma insulin levels based on previous work [342].

4.2 Research design and methods

4.2.1 Screening visit

Healthy people were recruited from the community (see Table 4.1). For the screening visit, participants were invited to come to the Menzies Institute for Medical Research clinical centre to confirm their eligibility by using medical questionnaires. Subjects were excluded from the study if they had any of the following characteristics: a history of smoking; current pregnancy; cardiac disease; history of severe liver disease; cancer within the last 5 years, history of drug or alcohol abuse; elective major surgery during the course of the study; or taking any medication known to affect glucose metabolism. Based on their fasting blood glucose (< 7.0mmol/L) and HbA1c (< 6.5%), participants were non-diabetic individuals. All participants gave written informed consent.

4.2.2 Clinic visit

After the screening visit, eligible participants were invited back to the Menzies Institute for Medical Research after an overnight fast. Participants refrained from exercise and alcohol for 48hr prior to testing and caffeine was omitted on the day of testing. Participants either underwent a MMC or an OGC and the following testing performed.

4.2.3 Oral glucose challenge (OGC) and mixed meal challenge (MMC)

OGC and MMC has been described in chapter 2.3.4. 24 participants competed OGC and 15 of them volunteered to participant in MMC.

4.2.4 Body composition

All participants underwent a whole body dual energy X-ray absorptiometry (DEXA) scan to assess body composition, in particular the amount of total body fat and trunk fat as described in Chapter 2.3.3.

4.2.5 Real-time contrast enhanced ultrasound (CEU)

Central (truncal) adipose tissue microvascular blood flow was assessed by CEU as described in Chapter 2.3.5. Adipose tissue microvascular responses were measured at baseline and then repeated 1hr following the OGC or MMC.

4.2.6 Image analysis

Digital image analysis was performed off-line using Qlab (Philips Medical Systems, Australia) as described in Chapter 2.3.6. MBV, β and MBF were calculated based on curve fitting described in Chapter 2.3.6.

4.2.7 Statistical analysis

Data are presented as the means ± SEM and statistics were performed using SigmaPlot (Systat Software, San Jose, CA, USA). Categorical variables are reported as numbers and percentages. Student's t-test was used to compare end point measurements between groups. When data were not normally distributed the Wilcoxon rank sum test was performed. For categorical variables a Fisher's exact test was performed. Two-way repeated measures ANOVA with Student-Newman-Keuls *post hoc* test was used to compare treatment groups over the time course of experiment. Pearson's bivariate correlations were used to evaluate associations. Spearman correlations were used to evaluate associations when data were not normally distributed. A value of $P < 0.05$ was considered as statistically significant.

4.3 Results

4.3.1 Characteristics of participants

The participant characteristics are presented in Table 4.2. There were no statistically significant differences in age, sex, anthropometrics, blood pressure or clinical chemistries between groups.

4.3.2 Glucose and insulin responses to oral glucose challenge (OGC) and mixed meal challenge (MMC)

Figure 4.1 shows the glucose and insulin responses to the OGC and MMC. Following the OGC and MMC, plasma glucose increased significantly and the glucose excursion was higher following the OGC when compare with MMC (Figure 4.1A). Consequently, the glucose area under the time course curve (AUC) during the OGC was markedly higher ($p < 0.001$) compared with the MMC (Figure 4.1B). However, the plasma insulin time course and the insulin AUC during the OGC and MMC were similar (Figure 4.1C and D).

4.3.3 Microvascular blood volume (MBV) and microvascular blood flow (MBF) responses to oral glucose challenge (OGC) and mixed meal challenge (MMC)

Figure 4.2 shows adipose tissue MBV and MBF responses at baseline and one hour following the OGC or MMC. Both the OGC and MMC increased MBV to a similar level above baseline ($p = 0.031$ and $p = 0.008$ versus baseline respectively) (Figure 4.2A). β was not significantly different at baseline or in response to the OGC and the MMC (Figure 4.2B). Both the OGC and MMC stimulated MBF to a similar extent but was only borderline significant ($p = 0.061$ and $p = 0.088$ versus baseline respectively) (Figure 4.2C).

4.3.4 Correlates to Microvascular blood volume (MBV) and microvascular blood flow (MBF) response to oral glucose challenge (OGC)

Correlations were conducted to determine associations with adipose tissue microvascular responses (MBV and MBF) to the OGC (Table 4.3) and the MMC (Table 4.4). Only baseline MBF was negatively associated with total body fat (%), truncal fat (%) and triglyceride (TG). Compared with correlates of adipose tissue MBV and MBF response to MMC (Table 4.4), baseline MBF was negatively associated with total body fat and trunk fat in MMC and OGC.

Table 4.1: Macronutrient composition of the oral glucose challenge (OGC) and mixed meal challenge (MMC).

	MMC	OGC
Energy (kJ)	1241	837
Protein (g)	21.7	-
Fat (g)	4.8	-
Carbohydrate (g)	41.0	50
Sugars (g)	25.1	50

Table 4.2: Characteristics of participants. Data are expressed as mean ± SEM.

Characteristics	OGC	MMC	P value
n	24	15	–
Age (years)	47 ± 2	46 ± 2	0.678
Sex	9F/15M	6F/9M	–
Height (cm)	172.6 ± 2.4	170.8 ± 2.8	0.309
Weight (kg)	76.8 ± 2.2	76.4 ± 2.5	0.699
BMI (kg/m^2)	25.8 ± 0.6	26.2 ± 0.8	0.366
Body Fat			
Total fat (%)	27.0 ± 1.8	28.1 ± 2.2	0.701
Trunk fat (%)	26.6 ± 1.6	27.8 ±2.1	0.670
Fasting glucose (mmol/L)	4.97 ± 0.14	4.63 ± 0.18	0.423
Fasting insulin (pmol/L)	45.0 ± 2.6	42.6 ± 3.5	0.777
HbA1c			
%	5.35 ± 0.05	5.33 ± 0.06	0.835
mmol/mol	35.00 ± 0.55	34.73 ± 0.73	0.776
Insulin Sensitivity Indices			
HOMA-IR	1.45 ± 0.11	1.27 ± 0.12	0.943
QUICKI	0.37 ± 0.01	0.37 ± 0.01	0.521
Blood Pressure			
SBP (mmHg)	123 ± 2	123 ± 2	0.708
DBP (mmHg)	76 ± 2	78 ± 2	0.944
Lipids			
Cholesterol (mmol/L)	5.10 ± 0.21	4.95 ± 0.23	0.922
Triglyceride (mmol/L)	0.88 ± 0.09	0.86 ± 0.07	0.477
HDL (mmol/L)	1.42 ± 0.06	1.49 ± 0.09	0.737
LDL (mmol/L)	3.19 ± 0.19	3.06 ± 0.21	0.893
FFA (mmol/L)	0.43 ± 0.03	0.40 ± 0.05	0.628

OGC: oral glucose challenge; MMC: mixed meal challenge.

Table 4.3: Correlates of adipose tissue MBV and MBF response to the OGC

Characteristics	Baseline MBV		OGC MBV		Baseline MBF		OGC MBF	
	r	*p*	r	*p*	r	*p*	r	*p*
Body fat								
Total fat (%)	-0.065	0.764	-0.054	0.807	**-0.454**	**0.029**	-0.145	0.504
Trunk fat (%)	-0.029	0.894	-0.050	0.822	**-0.504**	**0.014**	-0.140	0.518
Metabolism								
Fasting glucose (mmol/L)	0.273	0.205	-0.047	0.831	0.200	0.357	0.258	0.231
Glucose AUC	-0.055	0.799	-0.088	0.689	0.132	0.542	0.146	0.501
Fasting insulin (pmol/L)	0.054	0.802	-0.071	0.743	-0.403	0.056	-0.055	0.799
Insulin AUC	0.274	0.192	-0.019	0.930	-0.391	0.058	-0.244	0.246
HbA1c (%)	0.117	0.588	0.232	0.288	0.129	0.551	-0.020	0.926
QUICKI	-0.067	0.761	0.132	0.549	0.258	0.231	-0.022	0.919
Fasting serum triglyceride	-0.011	0.959	-0.132	0.542	**-0.508**	**0.014**	-0.180	0.407
Fasting plasma FFA	0.003	0.987	0.029	0.896	-0.341	0.110	-0.347	0.103
Blood pressure								
SBP (mmHg)	0.311	0.149	-0.120	0.584	0.163	0.452	0.095	0.663
DBP (mmHg)	0.133	0.539	-0.118	0.592	-0.106	0.623	0.046	0.830

Pearson's correlation was used between normally distributed variables. Spearman correlation was used if any of the variables were not normally distributed. Bold indicate significant correlations. MBV: Microvascular blood volume; MBF: microvascular blood flow; OGC: oral glucose challenge.

Table 4.4: Correlates of adipose tissue MBV and MBF response to the MMC

Characteristics	Baseline MBV		MMC MBV		Baseline MBF		MMC MBF	
	r	*p*	r	*p*	r	*p*	r	*p*
Body fat								
Total fat (%)	-0.348	0.204	0.124	0.660	**-0.713**	**0.002**	-0.154	0.585
Trunk fat (%)	-0.369	0.176	0.049	0.863	**-0.654**	**0.008**	-0.191	0.496
Metabolism								
Fasting glucose (mmol/L)	-0.211	0.441	-0.264	0.332	0.154	0.575	-0.446	0.092
Glucose AUC (mmol/L.120min)	-0.007	0.980	-0.118	0.676	0.154	0.575	-0.385	0.156
Fasting insulin (pmol/L)	**-0.540**	**0.038**	-0.365	0.181	-0.507	0.052	-0.197	0.482
Insulin AUC (mmol/L.120min)	0.018	0.944	-0.100	0.714	0.136	0.620	0.093	0.734
HbA1c (%)	0.078	0.783	-0.061	0.828	0.178	0.514	-0.199	0.477
QUICKI	**0.622**	**0.013**	0.400	0.140	0.429	0.107	0.154	0.584
Fasting serum triglyceride (mmol/L)	-0.311	0.259	-0.374	0.170	-0.302	0.263	**-0.626**	**0.013**
Fasting plasma FFA (mmol/L)	0.282	0.309	0.057	0.839	0.129	0.639	-0.304	0.270
Blood pressure								
SBP (mmHg)	0.146	0.604	0.288	0.299	0.189	0.489	0.227	0.415
DBP (mmHg)	0.385	0.156	**0.703**	**0.003**	0.271	0.319	0.261	0.069

Pearson's correlation was used between normally distributed variables. Spearman correlation was used if any of the variables were not normally distributed. Bold indicate significant correlations. MBV: Microvascular blood volume; MBF: microvascular blood flow; MMC: mixed meal challenge. (Data from Chapter 3 Table 3.3)

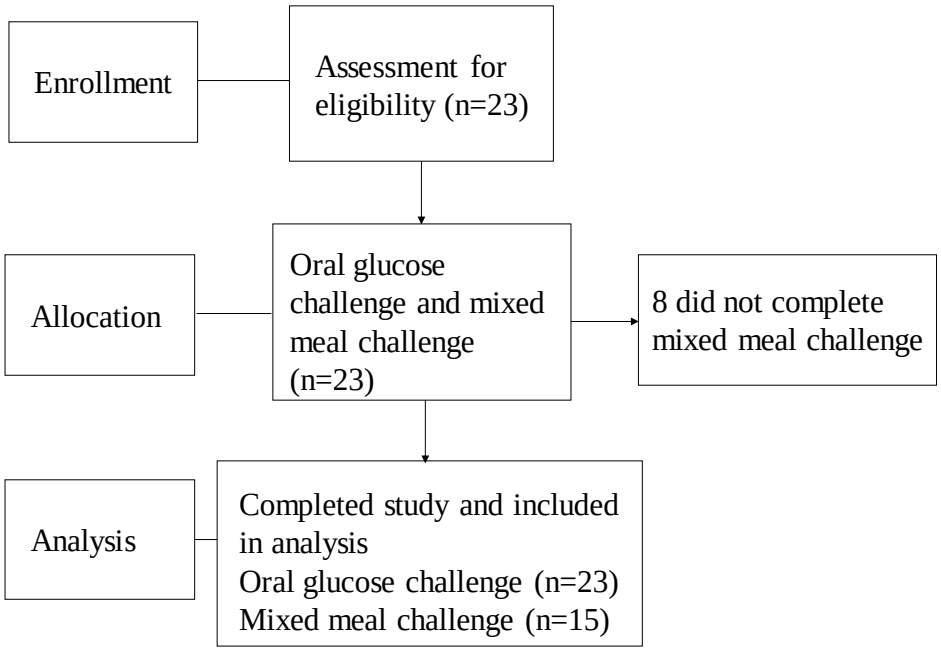

Figure 4.1: Flowchart of the number of participants screened, excluded, studied, and analysed.

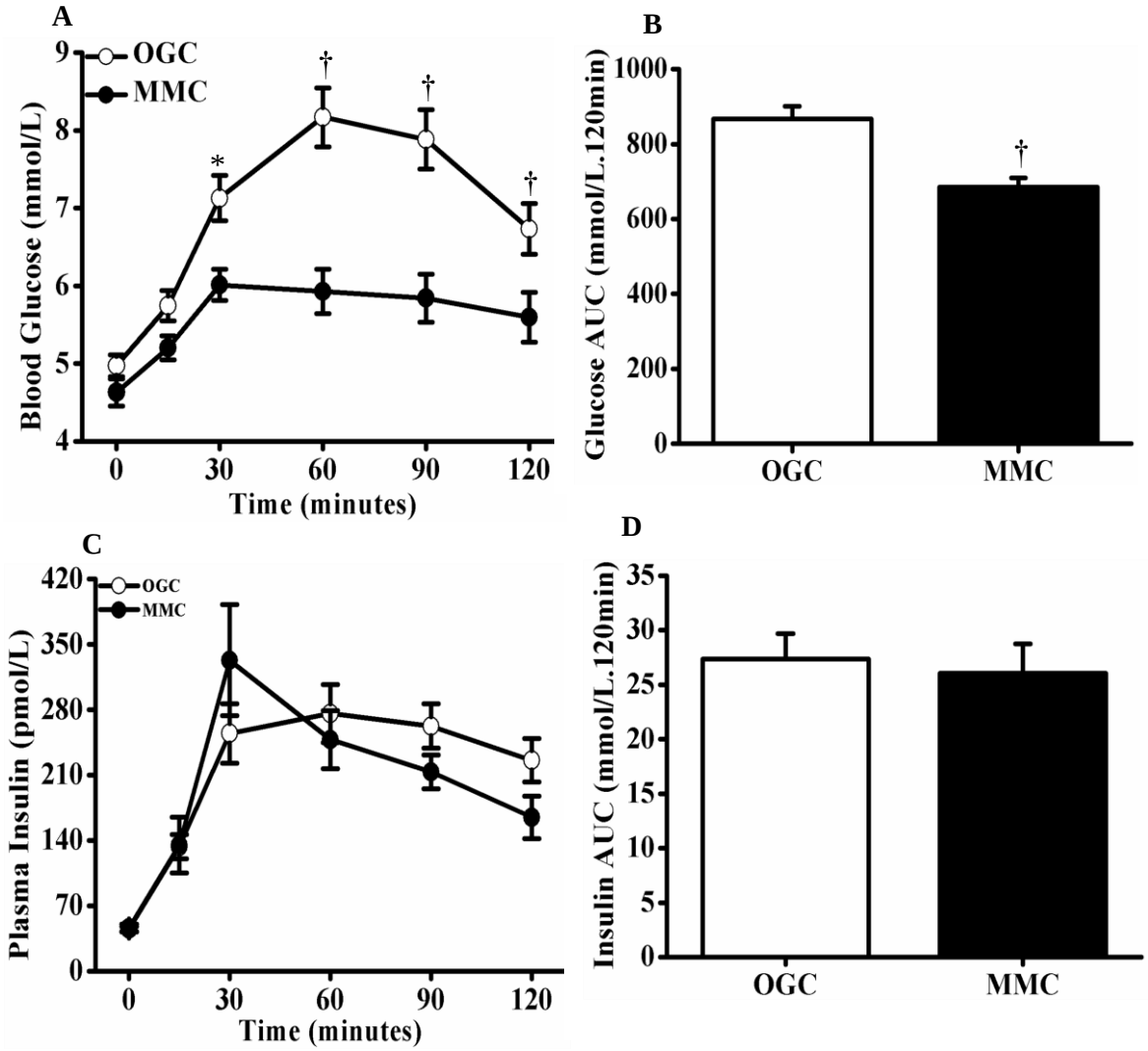

Figure 4.2: Plasma glucose and insulin levels in response to an OGC and a MMC. Plasma glucose (A) and insulin (C) time course in response to an OGC and MMC, and 2-hr glucose (B) and insulin (D) area under the curve during an OGC and MMC. Data are means ± SEM for each group. Repeated-measures two-way ANOVA was used to determine if there were differences between treatment groups over the time course of the experiment, or Student's t-test (or Wilcox Rank Sum Test if data not normally distributed) was used for single point measurements. When a significant difference was found, pairwise comparisons by the Student–Newman–Keuls test was used to determine treatment differences. *$P < 0.05$ vs. MMC; †$P < 0.01$ vs. MMC. MBV: Microvascular blood volume; MBF: microvascular blood flow; OGC: oral glucose challenge; MMC: mixed meal challenge.

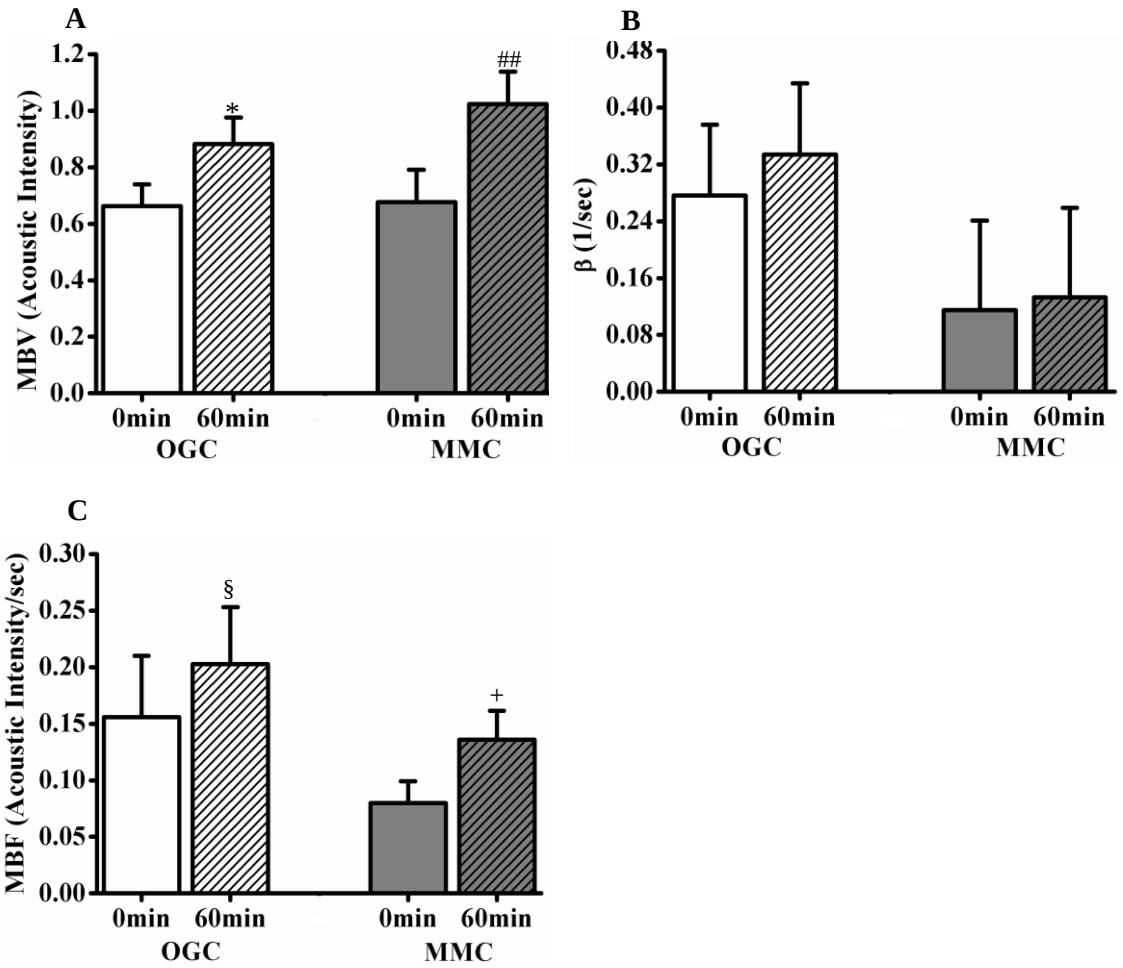

Figure 4.3: Adipose tissue microvascular responses to an OGC and MMC in healthy people. MBV (A), β (B) and MBF (C) at time 0 min (baseline) and 1-hr after OGC and MMC. Data are means ± SEM for each group. Repeated-measures two-way ANOVA was used to determine if there were differences between treatment groups over the time course of the experiment. When a significant difference was found, pairwise comparisons by the Student–Newman–Keuls test was used to determine treatment differences. $^{*}P < 0.05$ vs. baseline OGC; $^{\#\#}P < 0.01$ vs. baseline MMC; $^{\S}P = 0.061$ vs. baseline OGC; $^{+}P = 0.088$ vs. baseline MMC. MBV: Microvascular blood volume; MBF: microvascular blood flow; OGC: oral glucose challenge; MMC: mixed meal challenge.

4.4 Discussion

The present study for the first time compared the adipose tissue microvascular responses to an OGC and a MMC. Despite different macronutrient profiles, and blood glucose excursions, the OGC and MMC produced a similar increase in MBV and MBF in adipose tissue of healthy people. Both OGC and MMC produced similar insulin responses at 1hr following ingestion suggesting that insulin may play an important role in controlling perfusion (and nutrient delivery) to the adipocyte after a meal.

Adipose tissue MBV has been previously reported to increases in response to a 75g load of glucose in healthy people [275]. While most studies in humans focus on the MBV [274-276], the contribution of other important components of the microcirculation in adipose tissue, such as β and MBF, have been ignored. The current study confirms that MBV increases in response to an oral glucose load in healthy people, but also demonstrates that microvascular velocity (β) is not affected. Importantly, this study has established for the first time in humans that adipose tissue microvascular responses (whether MBV, β or MBF) are similar between the MMC and OGC despite very different macronutrient profiles and glycaemic loads.

The OGC produced a much higher blood glucose excursion when compared to the MMC. This is interesting because the vascular endothelium is susceptible to damage during prolonged hyperglycaemia and a growing body of literature suggests that acute hyperglycaemia can also impair vascular function in healthy people [352-354]. Ingestion of glucose (50g glucose) impairs brachial artery flow mediated dilation to a similar extent as a high glycemic-index meal (50g carbohydrate) when compared to a low glycemic-index meal (50g carbohydrate) [352]. Notably, it has been reported that the microvascular response to insulin is switched from dilation to constriction by the presence of hyperglycaemia [340]. Acute hyperglycaemia has been observed to diminish endothelial vascular responsiveness in healthy humans via activation of PKC [353] or reduced NO bioavailability [354]. Animal and cell culture studies have also demonstrated a direct effect of high glucose exposure to augment production of vasoconstrictors such as endothelin-1 [355] and prostanoids [356]. Given that pre-capillary arterioles are responsible for the regulation of capillary networks in all tissues,

it is perhaps surprising that OGC did not impair the microvascular responses under similar levels of hyperinsulinemia.

Triglycerides are reported to be negatively associated with total ATBF [100, 234]. The current study shows that baseline MBF in adipose tissue is negatively associated with triglyceride levels. This is interesting as it highlights the possibility that interventions to stimulate ATBF may have remarkable influence on reducing hypertriglyceridemia. This relationship was only observed in the post-prandial state in the group allocated to the MMC. This is an interesting observation given that the correlation was based on baseline levels of MBF and TG. Although TG levels were not statistically different between groups, the MMC group had a narrower range of fasting TG levels, which may explain the lack of association between TG and MBF in this group during fasting conditions. However, MBF was negatively associated with TG levels (whether fasting or post-prandial) in both OGC and MMC groups. Different ranges in plasma insulin, HOMA-IR and BP may also explain the different levels of significance with the associations with MBF and MBF in the MMC versus OGC groups. However a common feature between the OGC and MMC group is negative relationship with obesity.

In Chapter 3, the degree of obesity was found to be a strong modifier of adipose tissue microvascular blood flow (Figure 3.4). In this chapter, baseline MBF was also negatively associated with total body fat and truncal fat regardless of whether the group were allocated to MMC or OGC. This is not a surprising finding given that others have demonstrated a similar relationship between bulk ATBF and obesity [235, 272, 345]. It is well established that with adipocyte hypertrophy, as occurs in obesity, there is a reduction in capillary density [346], so, the greater degree of obesity similarly affects the ability to increase adipose tissue microvascular blood flow.

Our previous study has shown that OGC, which raised plasma insulin levels to a similar extent as the MMC, impaired microvascular responses (both MBV and MBF) in skeletal muscle of healthy individuals [351]. However, the present study provided that the OGC and MMC produced a similar increase in MBV and MBF in adipose tissue of healthy people. This may suggest that the mechanism of stimulating microvascular blood flow in skeletal muscle and adipose tissue is different.

In summary, the current chapter has demonstrated that 1) insulin (rather than the composition of the meal) is a key regulator of adipose tissue microvascular blood flow, 2) acute hyperglycaemia does not impair adipose tissue microvascular blood flow and 3) the degree of obesity affects the ability to increase adipose tissue microvascular blood flow. A limitation of the study is that a cross-over randomised study design was not used to compare the adipose tissue microvascular responses to an OGC and a MMC. It is now important to do follow up studies by matching total macronutrient profile (with the exception that one MMC contains glucose only as the source of carbohydrate whereas the other MMC contains complex carbohydrates) to see if similar observations are made with two different diets with different glycaemic indexes.

Chapter 5: Impairments in adipose tissue microcirculation in type 2 diabetes assessed by real-time contrast-enhanced ultrasound

5.1 Introduction

Adipose tissue is a highly dynamic endocrine organ, capable of secreting a number of hormones and pro-inflammatory mediators that regulate appetite, energy metabolism, and insulin action [357]. The dysregulation of normal adipose tissue function, as is the case with obesity, leads to exaggerated release of FFAs and pro-inflammatory cytokines into the circulation resulting in ectopic lipid deposition, low-grade chronic inflammation, and IR [117].

There has been renewed interest over the past decade in the effect of blood flow on metabolism and inflammation in adipose tissue in obesity, IR and T2D [122, 233-235, 237]. Blood flow in adipose tissue is important for delivery of macronutrients (e.g. glucose and triglycerides/lipoproteins), hormones (e.g. insulin) and oxygen to the adipocyte. Conversely, blood flow through adipose tissue also enables release of hormones (e.g. adiponectin) and metabolites (e.g. FFAs) from the adipocyte into the general circulation [232]. Impaired blood flow in adipose tissue has been proposed to cause hypoxia, resulting in tissue remodelling and the recruitment and activation of macrophages, leading in turn to amplified release of pro-inflammatory cytokines [107, 108]. In addition, impaired ATBF may also reduce nutrient exchange (e.g. glucose and triglycerides/lipoproteins) and hormone (e.g. insulin) delivery after a meal , leading to whole body IR [100].

Chapter 3 and 4 demonstrated that adipose tissue MBV and MBF were elevated in the post-prandial state of healthy subjects and this effect was blunted with a greater degree of obesity and IR (assessed by surrogate markers of IR). Whether adipose tissue MBV, β and MBF are impaired in people with T2D has not been investigated. Finally, whether impairments in microcirculation in adipose tissue are linked to the metabolic syndrome (the degree of adiposity, inflammation, blood pressure, IR, glucotoxicity and dyslipidemia) remains to be determined. The aim of the current study was to characterise adipose tissue microvascular responses in healthy people and those with

T2D using CEU, and establish whether there is an adipose tissue metabolic-linked microvascular phenotype in obesity and T2D.

5.2 Research design and methods

5.2.1 Screening visit

Healthy controls and people with T2D were recruited from the community (see Table 5.1 for their baseline characteristics). Participants with T2D had a previous clinical diagnosis by a doctor. During screening, participants were invited to the Menzies Institute for Medical Research Clinical Centre to establish eligibility by using medical questionnaires. Exclusion criteria from the study were: a history of smoking, current pregnancy, cardiac disease, history of severe liver disease, history of drug or alcohol abuse, cancer in the past 5 years, or major elective surgery during the course of the study. All participants provided written informed consent.

5.2.2 Clinic visit

After the screening visit, 24 healthy controls (9F/15M) and 21 subjects with T2D (8F/13M) were invited back after an overnight fast for testing. Participants refrained from exercise and alcohol for 48hrs prior to testing. Caffeine was omitted on the day of testing. Subjects with T2D refrained from taking their oral diabetes medication for 48hrs prior to testing. This is because these medications could affect results especially blood glucose levels. All participants were subjected to the following tests.

5.2.3 Body composition

Following height and weight assessment, body composition was determined by dual-energy X-ray absorptiometry (DEXA) as described in Chapter 2.3.3.

5.2.4 Oral glucose challenge (OGC)

Oral glucose challenge has been described in chapter 2.3.4.

5.2.5 Real-time contrast enhanced ultrasound (CEU)

Central (truncal) adipose tissue microvascular blood flow was assessed by CEU as described in Chapter 2.3.5. Background adipose tissue signal in the absence of contrast was identical between groups (healthy controls = 2.1±0.1 AI; T2D = 2.0±0.1 AI; p=0.417).

The arterial concentration of microbubbles was assessed by imaging the brachial artery at an infusion rate of 0.5ml/min to avoid signal saturation. When the acoustic intensity was scaled up to the dose infused for adipose tissue imaging, healthy controls and T2D had similar arterial levels (96.6±6.3 AI versus 87.4±7.4 AI, p=0.348).

5.2.6 Image analysis

Digital image analysis was performed off-line using Qlab (Philips Medical Systems, Australia) as described in Chapter 2.3.6

5.2.7 Inflammatory cytokines

Measurement of inflammation cytokines has been described in chapter 2.3.7.

5.2.8 Statistical analysis

Data are presented as the means ± SEM and statistics were performed using SigmaPlot (Systat Software, San Jose, CA, USA). Student's t-test was used to compare end point measurements between controls and T2D. When data were not normally distributed the Wilcoxon rank sum test was performed. For categorical variables a Fisher's exact test was performed. Two-way repeated measures ANOVA with Student-Newman-Keuls *post hoc* test was used to compare treatment groups over the time course of experiment. Pearson's bivariate correlations were used to evaluate associations. Spearman correlations were used to evaluate associations when data were not normally distributed. A value of $p < 0.05$ was considered as statistically significant.

5.3 Results

5.3.1 Baseline characteristics of subjects

The baseline characteristics of participants are presented in Table 5.1. Subjects with T2D had significantly higher body weight, BMI, total body fat (%), trunk fat (%), fasting blood glucose, fasting plasma insulin, HbA1c, HOMA-IR, systolic blood pressure (SBP), diastolic blood pressure (DBP), serum triglyceride and plasma FFA, and lower HDL and QUICKI (quantitative insulin sensitivity check index) when compare to control participants. Interestingly, LDL was significantly lower in people with T2D compared with healthy controls and is most likely reflective of greater statin use in this group. Medications and co-morbidities are also shown in Table 5.1.

5.3.2 Glucose and insulin responses to oral glucose challenge (OGC)

Blood glucose levels in people with T2D were significantly higher than controls at every time point (Figure 5.1A). Accordingly, the glucose area under curve (AUC) over the 2hrs in the T2D cohort was significantly higher ($p < 0.001$) compared with controls (Figure 5.1B). While fasting insulin levels were higher in the people with T2D, there were no differences in the time-course of plasma insulin levels during the OGC. As such, the insulin AUC for control subjects and those with T2D were also similar (Figure 5.1D).

The T2D cohort had elevated fasting insulin levels (Figure 5.1 and Table 5.1) but similar insulin response to the OGC when compared to healthy controls. This indicates that the T2D participants have some form of pancreatic dysfunction, as is the case with most people with T2D [358], but not considered late phase T2D. The T2D patients also did not have the additional complications of T2D that are more common in late phase T2D (e.g. self-reported nephropathy, retinopathy, neuropathy, or a history of heart attack or stroke).

5.3.3 Microvascular blood volume (MBV) and microvascular blood flow (MBF) responses to oral glucose challenge (OGC)

Figure 5.2 shows examples of adipose tissue contrast enhanced CEU images showing intensity of microbubbles before and 1hr-post OGC in healthy controls (Figures 5.2A and B, respectively) and those with T2D (Figures 5.2D and E, respectively). Corresponding curve fits after a destructive pulse of ultrasound in controls (Figure 5.2C) and those with T2D (Figure 5.2F) are also presented.

Figure 5.3 represents the averaged adipose tissue MBV, β and MBF values at baseline and 1 hour into a 50g OGC in control subjects and those with T2D. There was no statistically significant difference in MBV between control and T2D subjects at baseline (Figure 5.3). However, adipose tissue MBV in controls was significantly elevated 1 hr after the OGC ($p = 0.020$) and this response was completely absent in the people with T2D, being significantly lower than controls at the same time point ($p = 0.009$) (Figure 5.3A). β was not significantly different between healthy and T2D at baseline or in response to the OGC (Figure 5.3B). In healthy controls, MBF was not significantly elevated post OGC. However, baseline MBF in T2D appeared lower than the control group ($p = 0.079$) and was significantly lower than controls 1hr into the OGC ($p = 0.011$; Figure 5.3C).

5.3.4 Pro-inflammatory cytokines

Pro-inflammatory cytokines measured by ELISA at baseline are shown in Figure 5.4. There were no statistically significant differences observed in TNF-α, IL-6, CRP, MCP-1, IL-1β or sVCAM-1 between control subjects and those with T2D (Figure 5.4).

5.3.5 Correlates of adipose tissue microvascular blood volume (MBV) and microvascular blood flow (MBF)

Correlations were conducted to determine associations with adipose tissue microvascular responses (MBV and MBF) for all subjects (Table 5.2). The data of the diabetes and control group combined for the correlations to provide a greater range of data distribution. These variables were classified into four groups: body fat composition,

metabolism, blood pressure, and inflammation. Baseline MBF, but not baseline MBV, was negatively associated with truncal fat (%). However, both MBV and MBF in response to the OGC were negatively associated with truncal fat (%).

All markers of metabolism were significantly associated with baseline MBF and OGC MBF, with the exception of FFAs which were only associated with OGC MBF. In summary, fasting blood glucose, glucose AUC during the OGC, HbA1c, fasting insulin, TG and FFA levels were negatively associated with baseline and/or OGC MBF. QUICKI (a surrogate marker of insulin sensitivity) correlated positively with MBF. QUICKI was the only metabolic variable that correlated with MBV, being positively associated with the MBV response to the OGC. Fasting TG and FFA levels correlated negatively with MBF but not MBV. Systolic blood pressure correlated positively with baseline MBV. Inflammation was not associated with adipose tissue MBV or MBF at rest or in response to the OGC.

Table 5.1: Characteristics of participants

Characteristic	Control	Type 2 diabetes	P value
n	24	21	-
Age (years)	47 ± 2	52 ± 2	0.157
Sex	9F/15M	8F/13M	1.000
Diabetes Duration (yr)	-	9 ± 5	-
Hypertension, n (%)	1 (4)	10 (48)	**0.001**
Hypercholesterolemia, n (%)	-	13 (62)	**<0.001**
Height (cm)	172.6 ± 2.4	171.5 ± 1.7	0.460
Weight (kg)	76.8 ± 2.2	94.6 ± 5.4	**0.001**
BMI (kg/m^2)	25.8 ± 0.6	31.7 ± 1.5	**<0.001**
Body Fat			
Total fat (%)	27.0 ± 1.8	32.2 ± 1.4	**0.030**
Trunk fat (%)	26.6 ± 1.6	34.2 ± 1.3	**0.001**
Fasting glucose (mmol/L)	4.97 ± 0.14	9.82 ± 0.71	**<0.001**
Fasting insulin (pmol/L)	45.0 ± 2.6	113.2 ± 14.4	**<0.001**
HbA1c			
%	5.35 ± 0.05	7.65 ± 0.34	**<0.001**
mmol/mol	35.0 ± 0.6	58.0 ± 4.4	**<0.001**
Insulin Sensitivity Indices			
HOMA-IR	1.45 ± 0.11	7.19 ± 1.12	**<0.001**
QUICKI	0.37 ± 0.01	0.30 ± 0.01	**<0.001**
Blood Pressure			
SBP (mmHg)	123 ± 2	133 ± 3	**0.006**
DBP (mmHg)	76 ± 2	85 ± 2	**0.003**
Lipids			
Cholesterol (mmol/L)	5.10 ± 0.21	4.65 ± 0.22	0.251
Triglyceride (mmol/L)	0.88 ± 0.09	1.89 ± 0.20	**<0.001**
HDL (mmol/L)	1.42 ± 0.06	1.23 ± 0.10	**0.013**
LDL (mmol/L)	3.19 ± 0.19	2.58 ± 0.17	**0.026**
FFA (mmol/L)	0.43 ± 0.03	0.58 ± 0.04	**0.009**
Medication, n (%)			
Metformin	-	20 (95)	**<0.001**
Sulphonurea	-	2 (10)	0.212
GLP-1 RA	-	2 (10)	0.212
DPP4 inhibitor	-	3 (14)	0.094
SGLT2 inhibitor	-	1 (5)	0.467
Insulin	-	2 (10)	0.212
ACEi/ARB	-	7 (33)	**0.003**
Diuretic	-	4 (19)	**0.040**
Ca^{2+} channel blocker	1 (4)	5 (24)	0.083
Statin	-	10 (48)	**<0.001**
Other	12 (50)	12 (57)	0.767

Data are mean ± SEM. Student's t-test (or Wilcoxon Rank Sum Test if data not normally distributed) was used to determine differences between groups when the data were continuous. The Fisher Exact test was used to compare categorical data. ACEi (angiotensin converting enzyme inhibitor), ARB (angiotensin receptor blocker), DPP4 (dipeptidyl peptidase 4), GLP-1 RA (glucagon-like peptide-1 receptor agonist), SGLT2 (sodium-glucose cotransporter 2).

Table 5.2: Correlates of adipose tissue MBV and MBF (control and T2D participants combined).

Characteristics	Baseline MBV		OGC MBV		Baseline MBF		OGC MBF	
	r	*p*	r	*p*	r	*p*	r	*p*
Body fat								
Total fat (%)	0.129	0.403	-0.221	0.149	-0.279	0.067	-0.286	0.060
Trunk fat (%)	0.193	0.209	**-0.298**	**0.049**	**-0.429**	**0.004**	**-0.381**	**0.011**
Metabolism								
Fasting glucose (mmol/L)	0.016	0.920	-0.286	0.06	**-0.362**	**0.016**	**-0.367**	**0.015**
Glucose AUC (mmol/L.120min)	-0.092	0.551	-0.240	0.116	**-0.424**	**0.004**	**-0.376**	**0.012**
Fasting insulin (pmol/L)	0.047	0.759	-0.278	0.068	**-0.453**	**0.002**	**-0.374**	**0.012**
HbA1c (%)	-0.041	0.792	-0.18	0.241	**-0.327**	**0.031**	**-0.398**	**0.008**
QUICKI	-0.037	0.809	**0.328**	**0.03**	**0.498**	**0.001**	**0.48**	**0.001**
Fasting serum triglyceride (mmol/L)	0.008	0.956	-0.279	0.067	**-0.499**	**0.001**	**-0.302**	**0.046**
Fasting plasma FFA (mmol/L)	0.098	0.525	-0.116	0.452	-0.226	0.139	**-0.322**	**0.033**
Blood pressure								
SBP (mmHg)	**0.335**	**0.026**	-0.080	0.608	0.058	0.707	-0.021	0.890
DBP (mmHg)	0.139	0.365	-0.159	0.303	-0.205	0.182	-0.065	0.672
Heart rate (bpm)	0.061	0.686	-0.280	0.062	-0.316	**0.035**	-0.299	**0.046**
Inflammation								
TNF-α (pg/mL)	0.106	0.493	0.003	0.985	-0.087	0.575	-0.120	0.436
IL-1β (pg/mL)	0.025	0.871	-0.059	0.703	0.124	0.419	-0.109	0.478
IL-6 (pg/mL)	0.136	0.378	-0.26	0.089	-0.02	0.896	0.065	0.672
CRP (ng/mL)	0.111	0.471	-0.08	0.604	-0.065	0.675	-0.098	0.525
MCP-1 (pg/mL)	0.118	0.443	-0.129	0.403	-0.209	0.171	-0.206	0.063
sVCAM-1 (ng/mL)	0.006	0.969	0.219	0.153	-0.030	0.843	0.098	0.525

Pearson's correlation was used between normally distributed variables. Spearman correlation was used if any of the variables were not normally distributed. Bold indicate significant correlations. MBV: Microvascular blood volume; MBF: microvascular blood flow; OGC: oral glucose challenge.

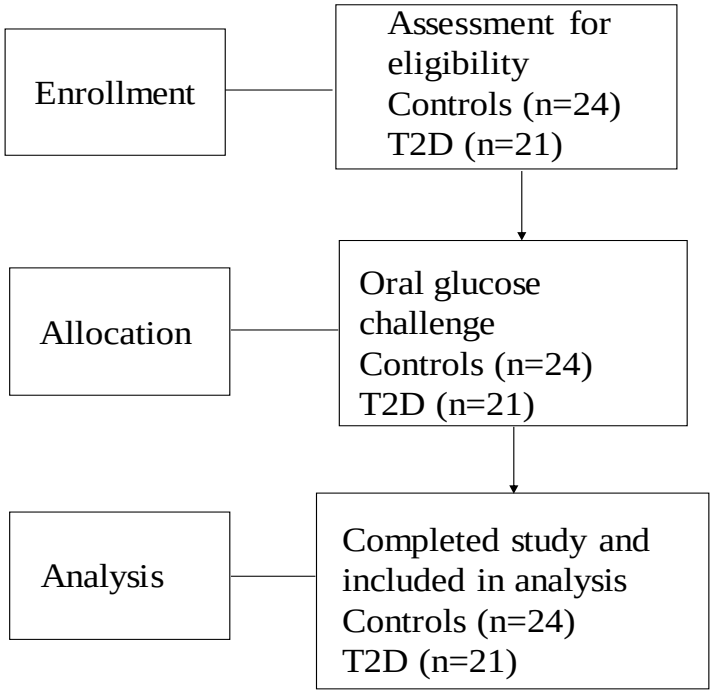

Figure 5.1: Flowchart of the number of participants screened, excluded, studied, and analysed.

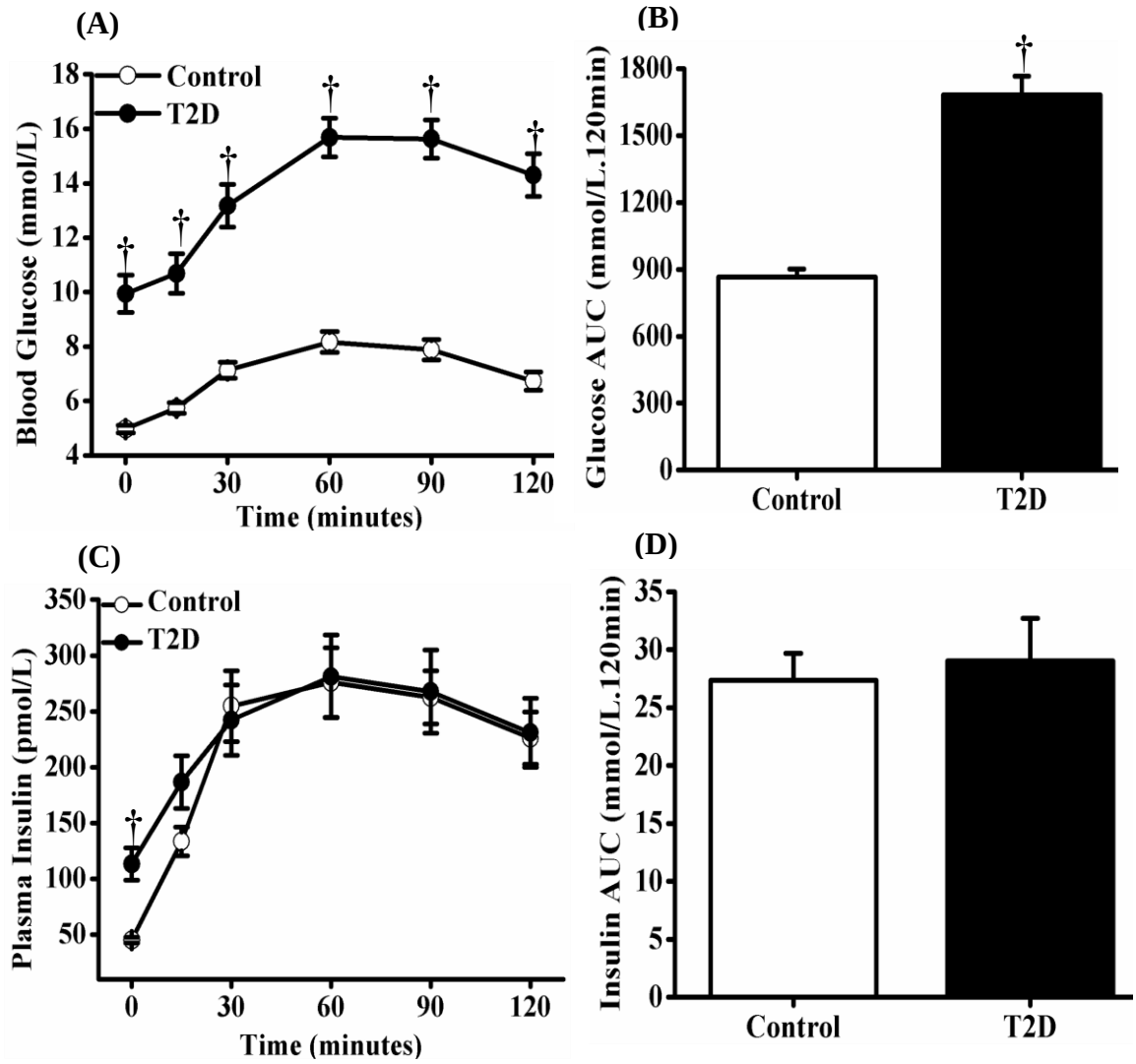

Figure 5.2: Blood glucose and insulin levels during the oral glucose challenge (OGC) (50g) in control (n=24) and type 2 diabetes (T2D, n=21) people. Blood glucose (A) and insulin (C) timelines in response to an OGC, and the calculated 2-hr glucose (B) and insulin (D) area under the curve (AUC) during the OGC. Data are means ± SEM for each group. Repeated-measures two-way ANOVA was used to determine if there were differences between treatment groups over the time course of the experiment, or Student's t-test (or Wilcoxon Rank Sum Test if data not normally distributed) was used for single point measurements. When a significant difference was found, pairwise comparisons by the Student–Newman–Keuls test was used to determine treatment differences. †$P < 0.01$ vs. control.

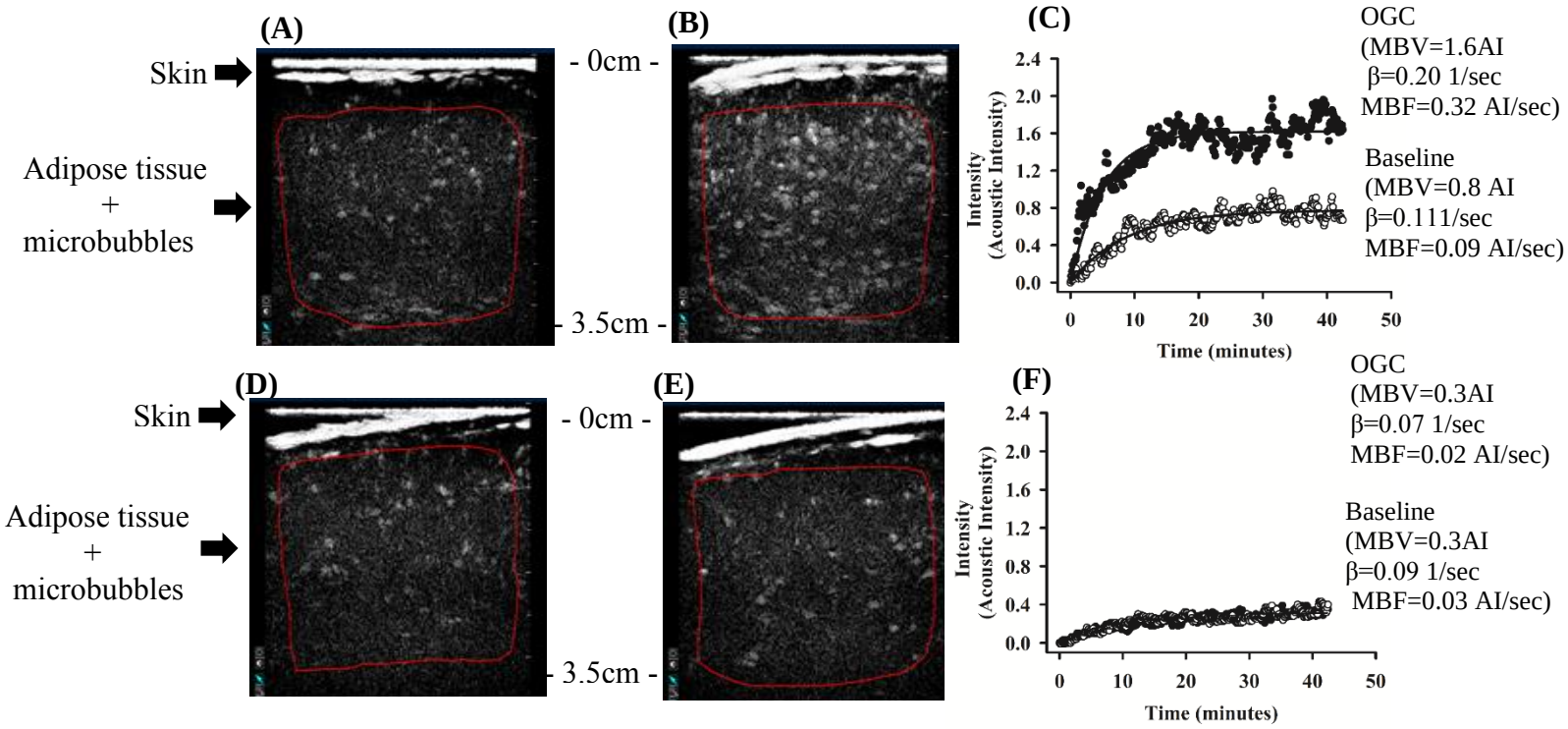

Figure 5.3: Examples of adipose tissue contrast enhanced images showing intensity of microbubbles before and after a 1hr OGC in controls (A and B, respectively) and those with type 2 diabetes (T2D, D and E, respectively)**.** The box in each figure represents the region of interest used for data analysis. Corresponding curve fits after a destructive pulse of ultrasound in controls (C) and those with T2D (F). MBV: Microvascular blood volume; MBF: microvascular blood flow; OGC: oral glucose challenge.

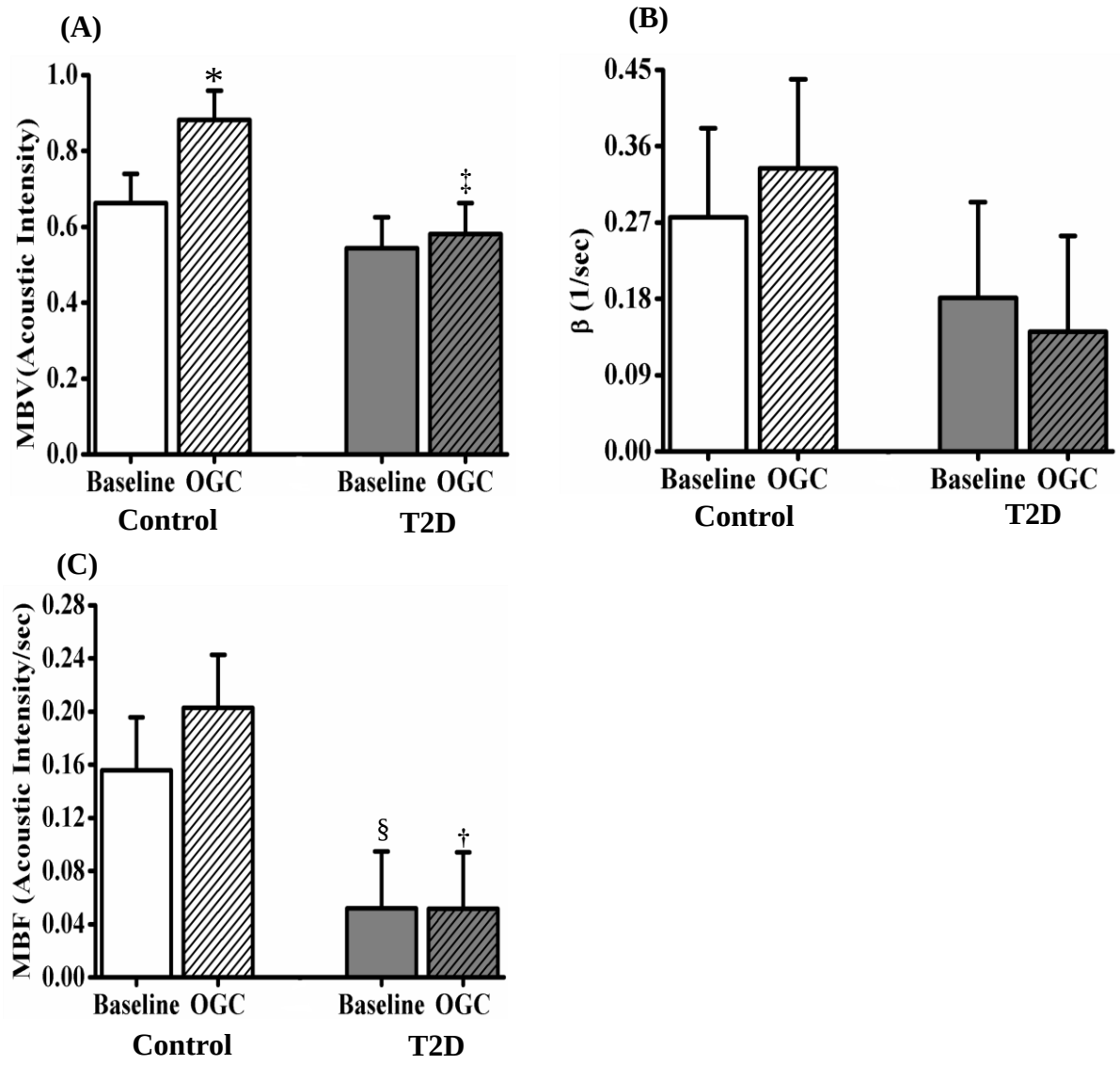

Figure 5.4: MBV, β and MBF responses to OGC in control (n=24) and type 2 diabetes (T2D, n=21) people. MBV (A), β (B) and MBF (C) at baseline and 1hr post-OGC. Data are means ± SEM for each group. Repeated-measures two-way ANOVA was used to determine if there were differences between treatment groups over the time course of the experiment. When a significant difference was found, pairwise comparisons by the Student–Newman–Keuls test was used to determine treatment differences. *$P < 0.05$ vs. control baseline; †$P < 0.05$ vs. control OGC; ‡$P < 0.01$ vs. control OGC; §$P = 0.079$ vs. control baseline. MBV: Microvascular blood volume; MBF: microvascular blood flow; OGC: oral glucose challenge.

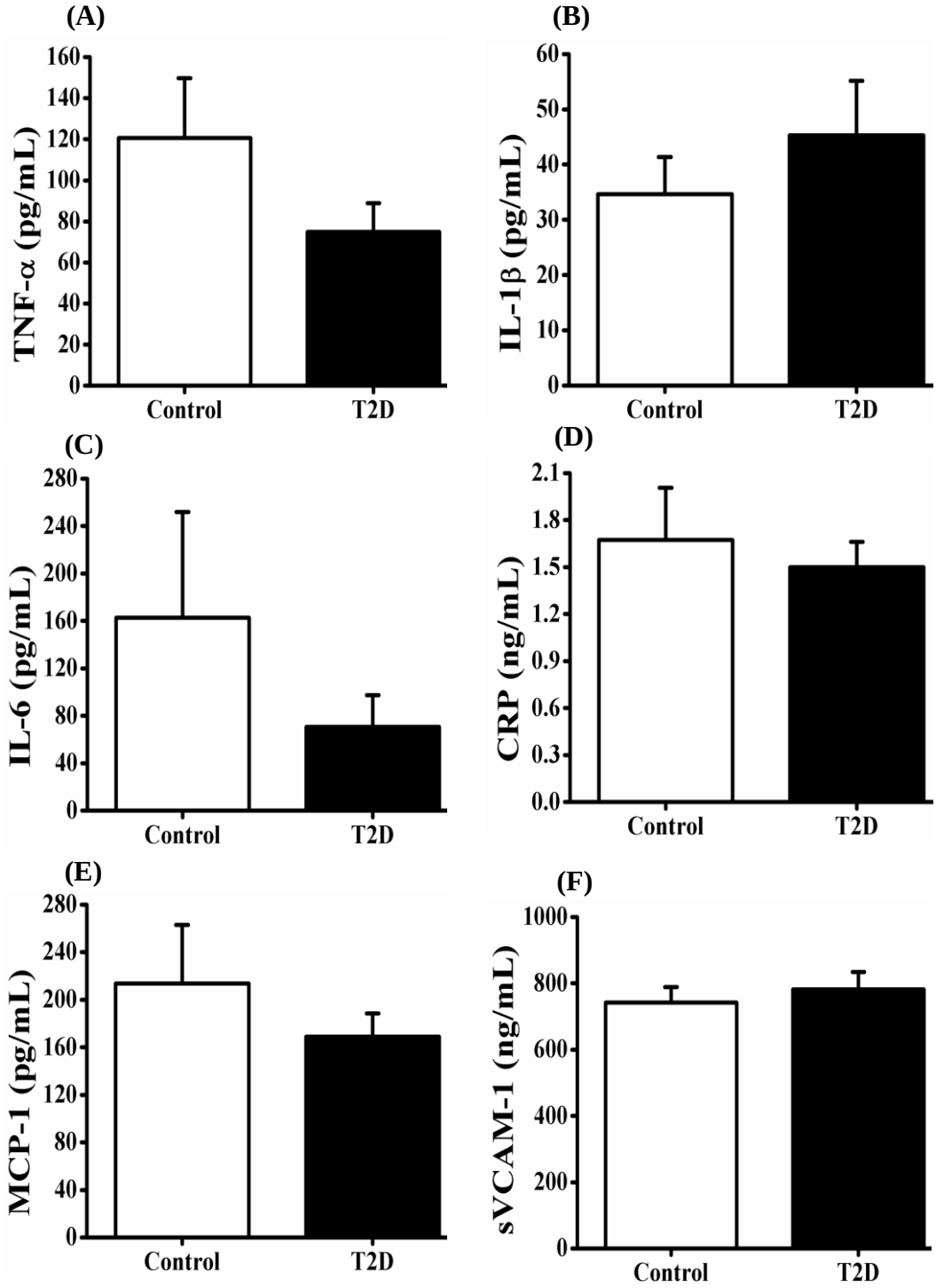

Figure 5.5: Fasting plasma TNF-α (A), IL-1β (B), IL-6 (C), CRP (D), MCP-1 (E) and sVCAM-1 (F) concentrations in control (n=24) and type 2 diabetes (T2D, n=21) people. Data are means ± SEM. Differences between treatment groups were assessed by Student's t-test (Wilcoxon Rank Sum Test if data not normally distributed).

5.4 Discussion

The present study demonstrates that adipose tissue MBF at rest and in response to ingestion of glucose is markedly impaired in individuals with T2D. Impaired adipose MBF responses were associated with higher amounts of body fat, increased TG and FFA concentrations, and glucose intolerance and hyperglycaemia. Surprisingly, no differences in circulating inflammatory markers between healthy and T2D individuals were observed. Therefore, it is concluded that impaired microvascular responses in adipose tissue of people with T2D are not conditionally linked to systemic inflammation, rather, are associated with IR, hyperglycaemia and dyslipidaemia.

There is only one study to date that has specially investigated microvascular responses in adipose tissue in humans with T2D. In that study adipose tissue MBV in response to a 75g oral glucose load was impaired in T2D, however MBF was not assessed [275]. The current study confirms that MBV increases in response to an oral glucose load in healthy people and that this response is not apparent in people with T2D. Importantly, this study has established for the first time in humans that adipose tissue MBF, rather than MBV, is more markedly impaired (by ~70%) in T2D both basally and in response to an OGC. This is important because a growing body of literature suggest that adipose tissue is hypoxic during obesity (which is common in T2D)[108] and these data suggest, that MBF is more closely linked to adipose tissue metabolic disturbances than MBV.

The current study focused on subcutaneous adipose tissue. Whether central (visceral) adipose tissue also displays the same microvascular abnormalities is yet to be confirmed. A limitation of the study was that the T2D participants were on a variety of medications when compared to the healthy controls (Table 5.1). Although diabetes-related medications were omitted for 48hrs prior to attending the clinic for testing, participants were still taking other medications (e.g. statins and anti-hypertensives) which may have contributed, at least in part, to variations in blood flow responses between groups.

In the current study, people with T2D had a significantly higher amount of total body and trunk fat compared with healthy controls (Table 5.1). Data from the current study indicate that the degree of adiposity, in particular truncal fat (%), is negatively associated with MBF and MBV measures, and the association was strongest with

baseline MBF (Table 5.2). These findings are not surprising given that a negative correlation was observed between body fat and MBF in the healthy group alone in Chapter 4 (Table 4.3). Thus, it appears that the degree of obesity rather than T2D *per se* is significantly associated with impaired adipose tissue microvascular responses. This is not a surprising finding given that others have demonstrated a similar relationship between bulk ATBF and obesity [235, 272, 345] and it is well established that with adipocyte hypertrophy, as occurs in obesity, there is a reduction in capillary density [346]. Belick et al established that obese and insulin resistant (*db/db*) mice have markedly larger adipocytes and impaired MBV and MBF [122]. However, the current study is the first to demonstrate a negative association between adiposity and both MBV and MBF in humans. It is intriguing that the relationship with MBF was stronger than MBV considering the reduction in capillary density might be expected to reduce MBV. It was anticipated that MBV would be lower in people with T2D and were surprised to find that their baseline MBV were similar to controls. This was also similarly observed by Tobin et al [275]. Histology to assess capillary density was not conducted in the current study and as such it has been speculated that 1) the capillary density was similar between controls and T2D or 2) there were fewer capillaries but a greater proportion were open at baseline in T2D. Given that a positive association between systolic blood pressure and baseline MBV was observed, one possibility is that the higher systolic blood pressure observed in T2D helps capillary patency at rest leaving less capillaries available for recruitment during the OGC. In chapter 3, the MBV in response to the MMC positively correlated with diastolic blood pressure (DBP) but not with systolic blood pressure (SBP). However, in chapter 5, this correlation disappeared when controls and T2D were combined together. Instead, we found a positive association between SBP and baseline MBV. It is important to note that the correction in the control cohort was stronger (r=0.703) than the combination of controls and T2D (r=0.335). The reasons for this are unknown but are important to follow-up in a larger trial. However, the greater reduction in MBF may have significant implications for the ability of adipose tissue to rapidly clear post-prandial nutrients such as triglycerides/lipoproteins in the obese state.

It is thought that as the adipocyte undergoes hypertrophy, insufficient microvascular blood supply to the adipocyte leads to hypoxia, macrophage recruitment and conversion of macrophages from an inactive state (M2) to an active state (M1) in which they are

reported to release TNF-α, IL-6, IL-1β and MCP-1 [85, 127]. Previous work has also demonstrated that activated macrophages are present in high numbers in subcutaneous adipose tissue of obese individuals and that weight loss decreases the number of activated macrophages [359]. It was proposed that the marked reduction in adipose tissue MBF in the T2D participants would be associated with a pro-inflammatory phenotype. However, no association between adipose tissue microvascular responses and inflammation was found. Further, no evidence of a systemic pro-inflammatory state in the T2D cohort was observed. This was confirmed by measuring systemic levels of well-known pro-inflammatory mediators (TNF-α, IL-6, MCP-1, CRP and IL-1β) and an additional marker of vascular inflammation (sVCAM). Tam and colleagues have reported that diet induced (28 day dietary intervention) weight gain and IR occurs in the absence of a significant inflammatory state in humans [360]. Other work suggests that inflammation is not conditionally linked with obesity-mediated IR [361, 362] and the current findings support this lack of association. However, adipose tissue levels of pro-inflammatory markers, histology on adipose tissue to assess the degree of macrophage recruitment and activation, or the assessment of adipose tissue oxygenation were not conducted in the current project. Inflammation may have been seen in this study if an older cohort or those with a higher amount of adiposity had been recruited. Nevertheless, in the study found no evidence of an adipose tissue microvascular-linked pro-inflammatory state in people with T2D.

Given that a microvascular-linked pro-inflammatory state was not observed in the T2D cohort, the next step was to determine whether impairments in microvascular responses in adipose tissue were related to glucotoxicity and/or dyslipidaemia, which are known to be associated with T2D. At baseline and in response to the OGC, MBF was significantly correlated with all insulin sensitivity/glucoregulatory function measures (fasting glucose, OGC AUC, fasting insulin, HbA1c, and QUICKI). In contrast, only QUICKI was associated with MBV. In chapter 4, the results have shown that insulin is a key regulator of adipose tissue microvascular blood flow. In this chapter, MBF response in people with T2D was markedly impaired, but the insulin response was very similar. Therefore, blood vessels in adipose tissue of people with T2D are insulin resistant. Belcik et al demonstrated that both MBV and MBF correlated negatively with fasting blood glucose and glucose AUC after an intraperitoneal insulin challenge in obese, insulin resistant mice [122]. Increases in skeletal muscle MBV in response to

insulin is important for muscle glucose disposal because it helps deliver glucose to the myocyte [277, 338, 342]. The current study demonstrates that MBF, rather than MBV in adipose tissue may be more important for glucoregulatory function and insulin sensitivity. Rates of glucose uptake in adipose tissue following a meal are smaller than those of skeletal muscle, so it is uncertain whether the improvement in adipose tissue MBF in healthy people following the glucose load promotes glucose uptake or whether excess circulating glucose in the people with T2D impairs microvascular function in adipose tissue. Rates of glucose uptake in the adipose tissue bed (arterio-venous glucose difference x flow or with isotopic glucose tracers) were not measured in the current study which will be required in future experiments to help address this question.

FFA and TG are reported to be negatively associated with total ATBF [100, 234]. The current study reports for the first time that MBF in adipose tissue has a similar negative association with FFA and TG levels. High blood viscosity due to elevated TG levels (ranging from ~0.2 to ~10mM by Intralipid infusion) has been demonstrated to affect coronary microvascular responses to hyperaemia [363]. Although people with T2D in the current study had elevated TG levels, this increase (1.89 ± 0.20 mM) is on the low end of the TG-blood viscosity range reported by Rim et al and as such is unlikely to affect blood viscosity in T2D subjectsin the present study. However, direct assessment of blood viscosity would be necessary to exclude this possibility. The direction of the association between TG and MBF is currently not known, however these data have implications for impaired microvascular responses in adipose tissue in the involvement of dyslipidaemia and ectopic fat accumulation.

In summary, the findings demonstrate an impairment in both MBV and MBF in adipose tissue of people with T2D. Chapter 4 demonstrated that the degree of obesity (independent of T2D) is a strong modifier of adipose tissue microvascular blood flow. However, there was only one association between microvascular responses and other metabolic parameters (blood glucose, insulin sensitivity, lipid profile) in the healthy group alone (Chapter 4) suggesting that T2D with concomitant obesity has additional detrimental impact on adipose tissue microvascular-linked lipidaemia and glycaemia, but not systemic inflammation. However, the direction of these associations are unknown and further experiments will help characterize the cause-and-effect. Improving microvascular function in adipose tissue may be a novel approach to prevent

pathogenesis of obesity related complications such as IR, dyslipidemia and glucotoxicity. A limitation of this study is that we did not match BMI between groups (control versus T2D).

Chapter 6: Metabolic benefits of resistance training in type 2 diabetes are not linked to improvements in adipose tissue microvascular blood flow

6.1 Introduction

Resistance training (RT) is recommended for people with T2D to improve overall cardiometabolic health [315, 316]. Specifically, RT improves insulin sensitivity, glycaemia, circulating lipids, body composition (i.e. increases muscle mass and reduces body fat) and is protective against cardiovascular disease (e.g. lowers blood pressure, aortic stiffness, and improves endothelial function) [364].

Keske and colleagues have recently demonstrated that in addition to these cardiometabolic benefits, six weeks of RT markedly enhances skeletal muscle microvascular blood flow (MBF) in T2D subjects in responses to an oral glucose challenge (OGC) [299]. Importantly, this enhanced skeletal muscle microvascular response was tightly linked to improvements in overall glycaemic control including reductions in fasting blood glucose and HbA1c levels, and improvements in glucose tolerance following an OGC [299]. Skeletal muscle is an important site for glucose disposal following a meal [365], and a greater MBF response following RT improves delivery of glucose and hormones (such as insulin) to the myocyte to improve glucose disposal [280]. These skeletal muscle microvascular-linked improvements with RT occurred independent of changes in body composition positioning the microvasculature in skeletal as an important regulator of overall glucose homeostasis.

Adipose tissue is also an important site for glucose disposal following a meal (albeit smaller than skeletal muscle) [366]. Perhaps more importantly, adipose tissue is a key site for the release of FFAs and a storage site for triglycerides [123]. Similar to skeletal muscle, adipose tissue has a dynamic microvascular blood supply to help promote the delivery and release of macronutrients such as oxygen, glucose and lipids [116]. Chapter 5 reported impairments in MBF and the recruitment of capillaries (MBV) in response to an OGC in central subcutaneous adipose tissue of people with T2D. These microvascular impairments in adipose tissue, in particular MBF, were associated with a greater degree of obesity, IR, hypertriglyceridemia, elevated plasma FFA levels, hyperglycaemia and glucose intolerance. Therefore improving microvascular function

in adipose tissue may be a novel approach to prevent pathogenesis of obesity related complications such as IR, dyslipidaemia and glucotoxicity.

While previous studies have shown exercise training improves microvascular flow (and consequently metabolic function) in skeletal muscle, there have been no studies assessing the impact of exercise training on adipose tissue microvascular responses in people with T2D. The aim of this study is to determine if six weeks of RT augments adipose tissue microvascular responses in sedentary people with T2D and whether this is paralleled by improvements in IR, hyperglycaemia and dyslipidaemia.

6.2 Research design and methods

The study was carried out in accordance with the Declaration of Helsinki as revised in 2008. The study protocol was approved by Tasmania Health & Medical Human Research Ethics Committee.

6.2.1 Screening visit

Sedentary (self-reported <30 min of moderate exercise per week) people with T2D were recruited through community advertisement. During screening, participants were invited to the Menzies Institute for Medical Research Clinical Centre to establish eligibility by using a medical questionnaire. Participants were included in the study if they were between 18 and 60 years of age, had a clinical diagnosis of T2D, and were normal weight to overweight (BMI 19 -35 kg/m^2). Participants were excluded from the study if they participated in any kind of resistance exercise or performed more than low-intensity walking. Additional exclusion criterion included having a BMI >35 kg/m^2 or a personal history of smoking, cardiovascular disease, stroke, myocardial infarction, uncontrolled hypertension (seated brachial blood pressure >160/100 mmHg), peripheral arterial disease, pulmonary disease, arthritis/muscular skeletal disease, malignancy within the past five years, or severe liver disease. A medical practitioner examined each participant to confirm eligibility to participate in an exercise program.

6.2.2 Clinic visit

After the screening visit, participants were invited back after an overnight fast. Participants refrained from exercise and alcohol 48 hr prior to testing and caffeine on the morning of the study. Diabetes medications were stopped for 48 hr prior to testing. Participants were asked to complete a physical activity questionnaire (IPAQ) to confirm eligibility that they were sedentary. All participants were subjected to the following tests.

6.2.3 Body composition

Subjects underwent a whole body scan to assess body composition before and after RT by dual-energy X-ray absorptiometry (DEXA) from soft tissue composition as described in Chapter 2.3.3.

6.2.4 Oral glucose challenge (OGC)

Oral glucose challenge (OGC) has been described in chapter 2.3.4. OGC was assessed before and after RT.

6.2.5 Real-time contrast enhanced ultrasound (CEU)

Central (truncal) subcutaneous adipose tissue microvascular blood flow was assessed by real-time CEU as described in Chapter 2.3.5. The reflow dynamics of microbubbles into adipose tissue microvasculature was assessed in real-time at baseline and then repeated 1hr following an OGC before and after RT.

6.2.6 Image analysis

Digital image analysis was performed off-line using Qlab (Philips Medical Systems, Australia) as described in Chapter 2.3.6

6.2.7 Resistance Training (RT) Intervention

The six-week RT programme used in this study was based on previous RT studies [293, 299]. RT was performed three days per week at the same time at a local fitness centre in Hobart, Tasmania, Australia (All Aerobic Fitness). The training regime was divided into a full body workout on Monday and Friday, with core and stability exercises on Wednesday. The full body workout used a mixture of free-weights and resistance machines. One set of each resistance exercise was performed to complete muscle failure (6-15 reps) and included in the order: leg press, lateral pull-down, chest press, weighted lunges, seated row, back fly, bicep curl, incline chest press, dumbbell shoulder press, leg extension, leg curl, dips, lateral shoulder raise, triceps extension, dumbbell deadlift, and push-ups. As for core and stability exercises, the workouts used a range of resistance-focused techniques such as dumbbell sit-ups, medicine ball toss, leg-lifts,

plank positions, burpees, and weighted farmer's walk, but they were not limited to these. These workouts were continually modified to match increased strength and fitness.

Each session was limited to one hour. All resistance exercises were recorded with the load incrementally increased [maintained between 65%-85% of calculated 1 repetition maximum (1RM)] as strength was increased to ensure progression. Participants were encouraged to drink water if they need before, during and after RT. Also, participants underwent a DEXA scan the week before returning to the clinic for repeated a series of cardiometabolic testing as described above.

6.2.7 Inflammatory cytokines/markers

Measurement of inflammation cytokines has been described in chapter 2.3.7.

6.2.8 Statistical analysis

Data are presented as the means ± SEM. Student's paired t-test was used to compare end point measurements between Pre-RT and Post-RT. When data were not normally distributed or Wilcoxon Signed Rank Test was performed. For all continuous variables, a two-way repeated measures ANOVA (interactions: time: 0 and 60 min group: pre-RT and post-RT) followed by a Student–Newman–Keuls post-hoc was performed. Significance was set at $p<0.05$. Tests were performed using SigmaStat™ statistical program (Systat Software, San Jose, CA, USA).

6.3 Results

6.3.1 Characteristics of subjects before and after RT

The characteristics of participant before and after RT are presented in Table 6.1. As expected, following six weeks of RT, participants had significant reductions in total body fat (p = 0.002) and trunk fat (p = 0.023). These changes in body composition occurred without changes in overall body weight or BMI. Fasting blood glucose (p = 0.006), HbA1c (p = 0.007), HOMA-IR (p = 0.005) and fasting serum triglyceride levels (p = 0.029) were significantly lower following RT, whereas fasting plasma insulin, QUICKI, blood pressure, total cholesterol, HDL, LDL and FFA were unaffected.

6.3.2 Blood glucose and insulin responses to the oral glucose challenge (OGC) before and after RT

Figure 6.1 shows the time course of blood glucose and plasma insulin levels before and after a 50 g OGC. Following RT, plasma glucose levels were significantly lower during the OGC except at 90 min (Figure 6.1A) and the area under the glucose time curve (Figure 6.1B) was significantly lower (p = 0.014). Plasma insulin levels during the OGC were significantly lower at 15, 30 and 60 min post-OGC (Figure 6.1C) and area under the insulin time curve was also significantly lower after RT (p = 0.036, Figure 6.1D).

6.3.3 Adipose tissue microvascular blood volume (MBV) and microvascular blood flow (MBF) responses to oral glucose challenge (OGC) before and after RT

Adipose tissue MBV and MBF responses to the OGC before and after RT are shown in Figure 6.2. Baseline MBV (p = 0.102), β (p = 0.885), and MBF (p = 0.225) did not improve following RT. Similarly, there were no significant changes in MBV, β or MBF responses to the OGC after six weeks of RT (Figure 6.2).

6.3.4 Effect of RT on circulating pro-inflammatory markers

Pro-inflammatory cytokines measured by ELISA before and after RT are shown in Figure 6.3. There was a significant increase in TNF-α and sVCAM-1 post-RT. However,

there were no statistically significant differences observed in IL-6, CRP, MCP-1, or IL-1β before and after RT.

Table 6.1: Characteristics of study participants before and after RT. Data expressed as Mean ± SEM (n=18).

Characteristics	Pre-RT	Post-RT	P value
Age (years)	52 ± 2	-	-
Sex	7F/11M	-	-
Diabetes Duration (years)	9 ± 1	-	-
Diabetes Medication			
Lifestyle only (%)	1 (6)	-	-
Metformin (%)	17 (94)	-	-
Sulphonylurea (%)	2 (11)	-	-
Insulin (%)	2 (11)	-	-
GLP-1 RA (%)	2 (11)	-	-
DPP4 inhibitor (%)	1 (6)	-	-
SGLT2 inhibitor (%)	1 (6)	-	-
Height (cm)	170.9 ± 1.88	-	-
Weight (kg)	94.69 ± 6.13	90.47 ± 3.88	0.421
BMI (kg/m^2)	31.94 ± 1.75	30.75 ± 1.02	0.596
Body Fat			
Total fat (%)	32.06 ± 1.57	31.14 ± 1.61	**0.002**
Trunk fat (%)	34.10 ± 1.45	33.09 ± 1.45	**0.023**
Fasting glucose (mmol/L)	10.23 ± 0.78	9.04 ± 0.69	**0.006**
Fasting insulin (pmol/L)	111.87 ± 15.43	98.42 ± 13.11	0.108
HbA1c			
%	7.78 ± 0.37	7.44 ± 0.34	**0.007**
Insulin Sensitivity Indices			
HOMA-IR	7.76 ± 1.24	5.72 ± 0.96	**0.005**
QUICKI	0.30 ± 0.01	0.31 ± 0.01	0.078
Blood Pressure			
SBP (mmHg)	132.8 ± 3.4	130.4 ± 2.7	0.388
DBP (mmHg)	84.1 ± 2.5	83.3 ± 2.2	0.602
Lipids			
Cholesterol (mmol/L)	4.69 ± 0.24	4.50 ± 0.23	0.260
Triglyceride (mmol/L)	1.82 ± 0.23	1.47 ± 0.016	**0.029**
HDL (mmol/L)	1.27 ± 0.11	1.28 ± 0.10	0.808
LDL (mmol/L)	2.60 ± 0.19	2.55 ± 0.20	0.734
FFA (mmol/L)	0.59 ± 0.05	0.56 ± 0.06	0.485

Student's t-test (or Signed Rank Test if data not normally distributed) was used to determine differences. ACEi (angiotensin converting enzyme inhibitor), ARB (angiotensin receptor blocker), DPP4 (dipeptidyl peptidase 4), GLP-1 RA (glucagon-like peptide-1 receptor agonist), SGLT2 (sodium-glucose cotransporter 2).

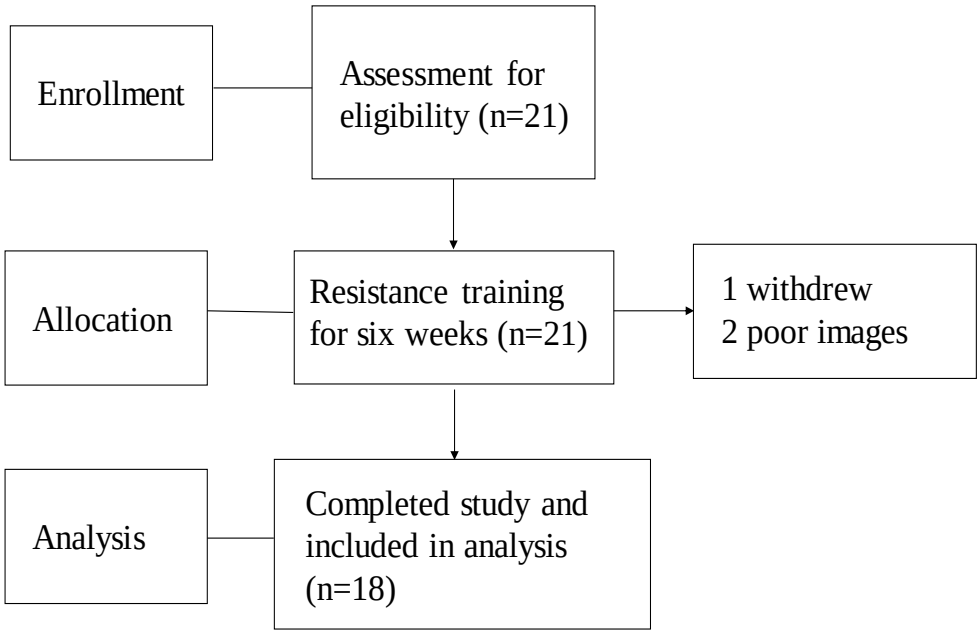

Figure 6.1: Flowchart of the number of participants screened, excluded, studied, and analysed.

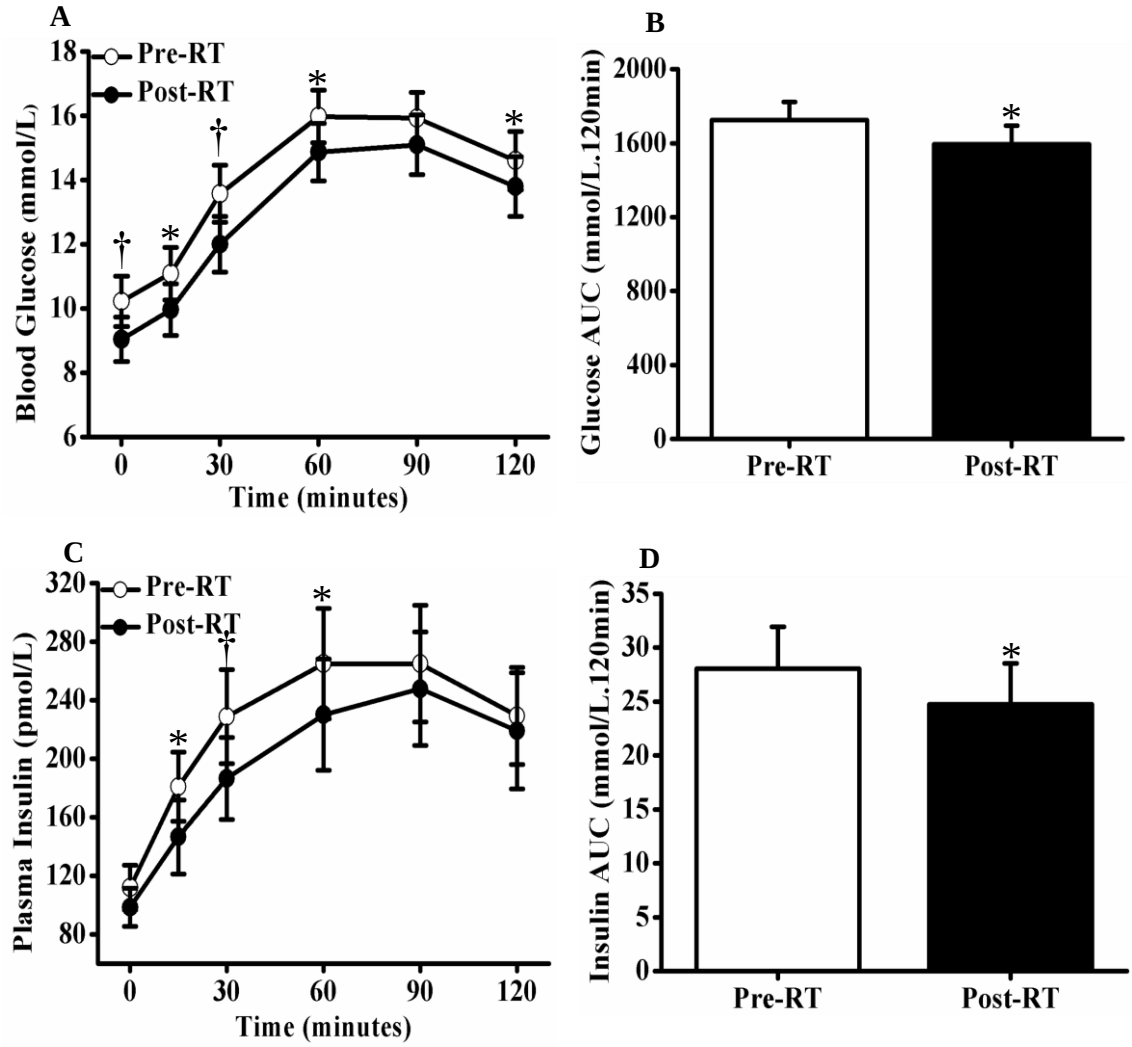

Figure 6.2: Blood glucose (A) and plasma insulin (C) timelines in response to an OGC, and 2-hr glucose (B) and insulin (D) area under the curve during a 50g OGC. Data are means ± SEM for each group. Repeated-measures two-way ANOVA was used to determine if there were differences between treatment groups over the time course of the experiment, or Student's paired t-test (or Signed Rank Test if data not normally distributed) was used for single point measurements. When a significant difference was found, pairwise comparisons by the Student–Newman–Keuls test was used to determine treatment differences. *$P < 0.05$ vs. control; †$P < 0.01$ vs. control.

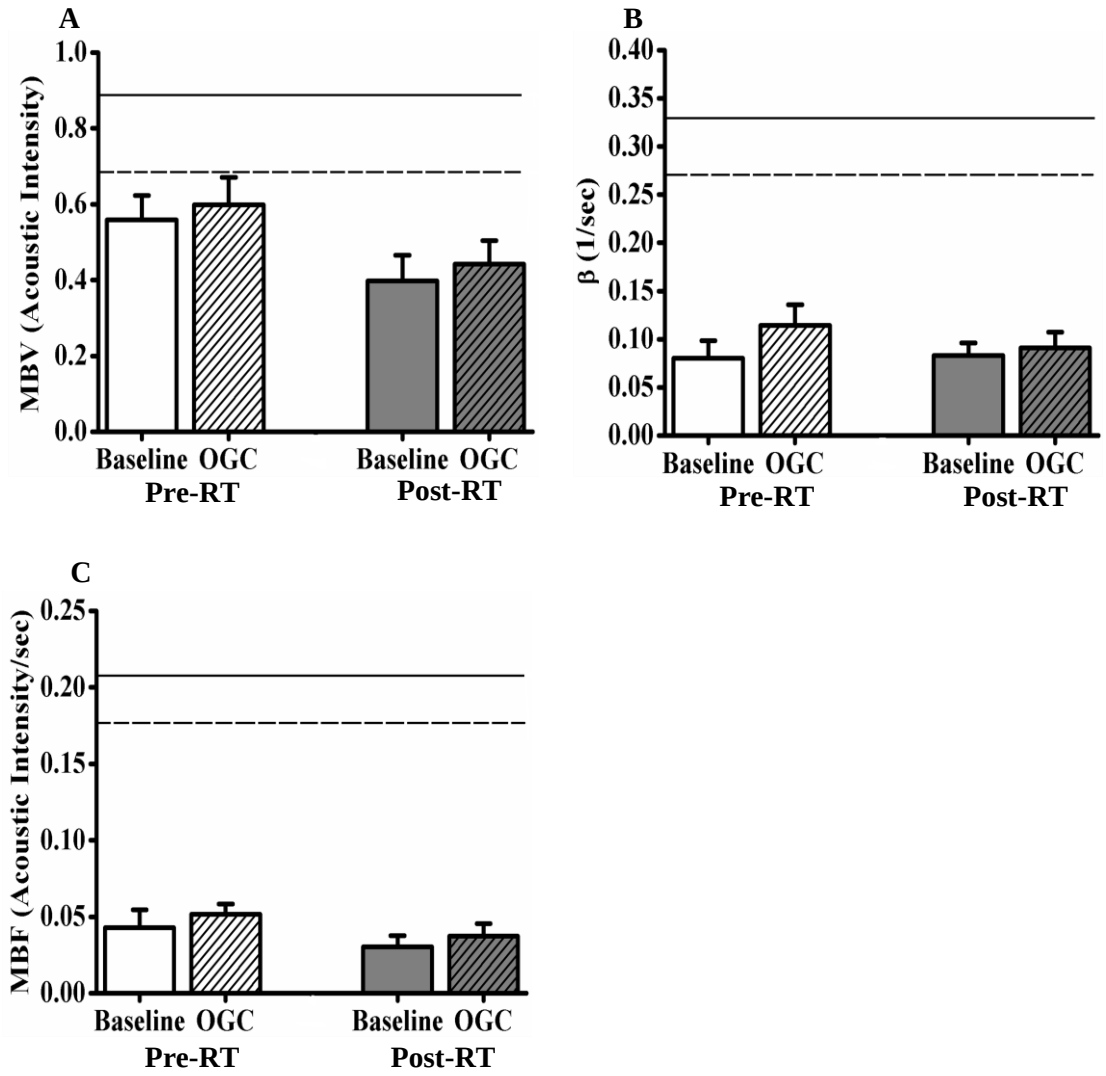

Figure 6.3: Adipose tissue MBV, β and MBF responses to OGC before and after RT in people with T2D. MBV (A), β (B) and MBF (C) at baseline (time 0-min) and after OGC (time 60-min). Data are means ± SEM for each group. Repeated-measures two-way ANOVA was used to determine if there were differences between treatment groups over the time course of the experiment. When a significant difference was found, pairwise comparisons by the Student–Newman–Keuls test was used to determine treatment differences. Dotted and solid lines represent baseline and post OGC responses in healthy people (data taken from Chapter 4). MBV: Microvascular blood volume; MBF: microvascular blood flow; OGC: oral glucose challenge.

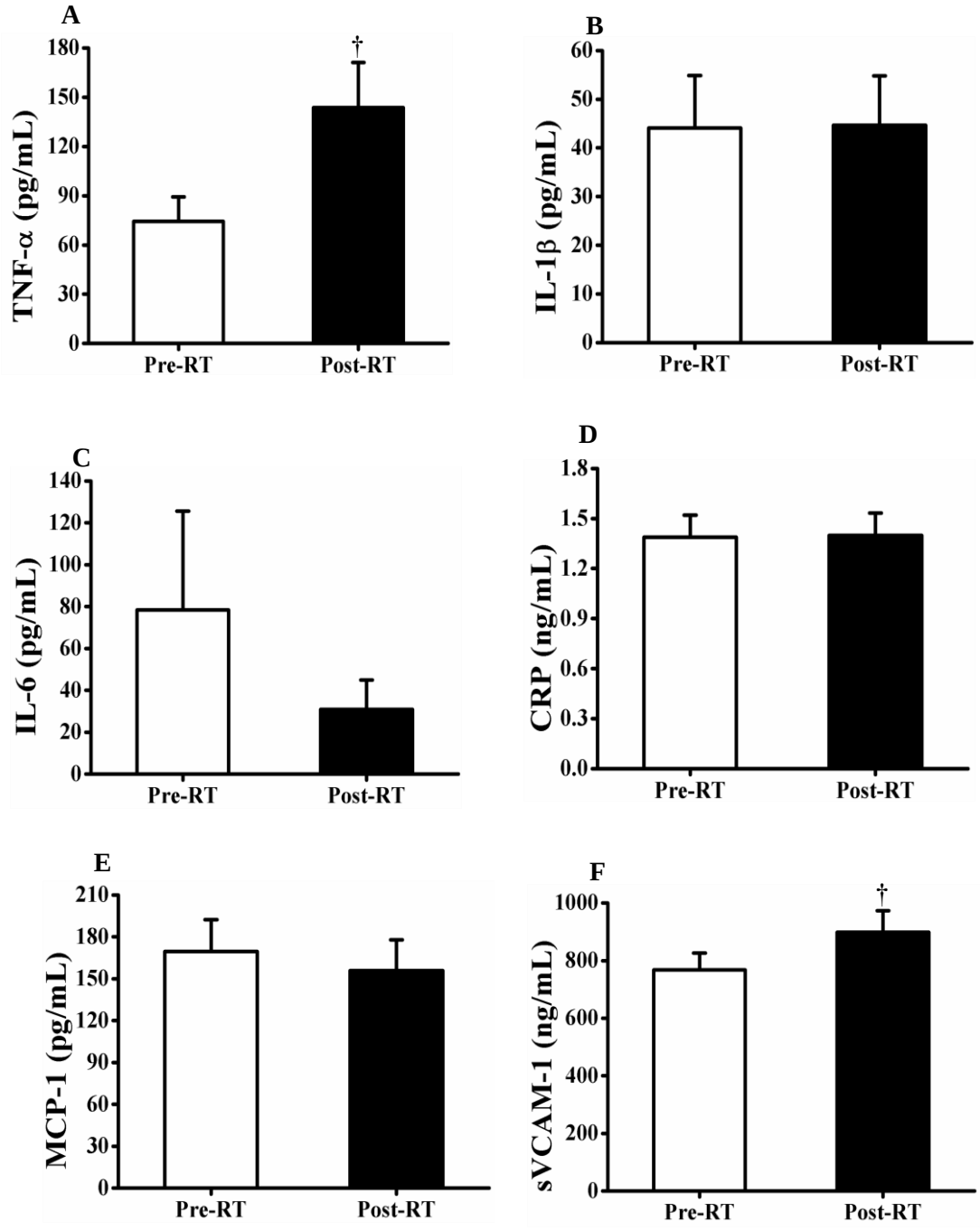

Figure 6.4: Fasting plasma TNF-α (A), IL-1β (B), IL-6 (C), CRP (D), MCP-1 (E) and sVCAM-1 (F) concentrations in type 2 diabetes (T2D, n=21) people. Data are means ± SEM. Student's paired t-test (or Wilcoxon Signed Rank Test if data not normally distributed) was used for single point measurements.

6.4 Discussion

This is the first study to assess the impact of exercise training on microvascular responses in adipose tissue of people with T2D. The study demonstrated that the well-known metabolic and anthropometric benefits of six weeks of RT are not paralleled by improvements in adipose tissue MBV, microvascular flow velocity (β) or MBF. These results are surprising given that poor adipose tissue microvascular responses, in particular MBF, are associated with a greater degree of adiposity, dyslipidaemia, and glucotoxicity. Therefore, it is concluded that six weeks of RT in people with T2D produced favourable effects of glycaemic regulation, circulating lipids and body composition, however these effects occurred without a concomitant increase in adipose tissue microvascular responses at rest or during an OGC. In chapter 4, our findings have shown that insulin is a key regulator of adipose tissue microvascular blood flow. In this chapter, MBV and MBF response before and after RT were not different, but the insulin responses were improved. There are a number of insulin sensitive tissues in the body (liver, adipose tissue and skeletal muscle. Therefore, the improvement in insulin levels of RT may be indicative of liver and skeletal muscle being more insulin sensitive rather than adipose tissue.

There are very few studies that have investigated the effects of chronic exercise interventions on human ATBF. To date, most studies on human ATBF, such as those of Frayn and colleagues [99, 101, 272] have used ^{133}Xenon washout which measures the disappearance of the isotope injected into adipose tissue where faster disappearance reflects higher blood flow in adipose tissue. Using this technique it has been reported that ATBF is higher in trained *versus* sedentary healthy individuals [327, 328]. Given this finding, it would be reasonable to assume that exercise training interventions would likewise increase ATBF, however the evidence so far is not clear. Sixteen weeks of endurance exercise training in young healthy lean men improves aerobic capacity by ~25%, but does not improve body composition (fat mass or lean muscle mass) or resting or epinephrine stimulated ATBF [329]. Similarly, 12 weeks of aerobic exercise training in healthy older women produced a significant increase in exercise capacity, but again, this improvement was not associated with changes in body composition or resting ATBF [330]. There have also been mixed findings regarding the impact of chronic

exercise training (12-16 weeks) on ATBF in overweight/obese individuals when assessed indirectly using microdialysis [331, 332]. This lack of association between chronic exercise training and ATBF may result from indirect blood flow measurements which are not assessing flow at the microvascular level (the critical site for nutrient exchange). In addition, previous studies have been conducted in healthy subjects where the microcirculation is already functioning normally. In contrast, people with T2D have impaired microvascular function, which in skeletal muscle, has been shown to improve with exercise training. Given this, it was hypothesised that microcirculation in adipose tissue may respond in a similar way and that resistance training may help to restore this impaired vascular function.

Over the past 15 years it has been demonstrated that microvascular blood flow is important in determining insulin's metabolic effects in skeletal muscle independent of changes in total limb blood flow [280, 299, 338, 342, 347, 367-369]. This was made possible in part with the adaptation of the CEU technique for skeletal muscle. In the present study use of a novel real-time CEU imaging to assess microvascular blood flow responses in adipose tissue. This is an important distinction from other techniques because nutrient exchange occurs at the microvascular level. The CEU technique has the capacity to isolate the measurement to the microcirculation and dissect different perfusion components – in particular, microvascular blood volume (MBV – the number of capillaries being perfused), microvascular flow velocity (β –the filling rate of the capillaries being perfused) and microvascular blood flow (MBF – which is the product of MBV and β) [280, 281]. Thus using this technique it was possible to dissect different adipose tissue microvascular responses in people with T2D and which components are altered following six weeks of RT. It was a surprise to find that adipose tissue microvascular responses (MBV, β and MBF) were not altered following RT.

There are several possibilities as to why there were not any significant improvements in adipose tissue microvascular responses in adipose tissue of people with T2D following RT. Firstly, the length of training may not have been sufficient to cause improvements in adipose tissue MBV or MBF at rest or during the OGC. This is particularly important given that it was observed in Chapter 5 that the degree of obesity is negatively associated with adipose MBV and MBF. Secondly, this type of exercise

training (RT rather than aerobic exercise) may not be sufficient to sensitize the microcirculation to respond to the OGC. Although it has been previously demonstrated that marked improvements in skeletal muscle MBF occur following six weeks of RT in people with T2D [299], the regulation of microcirculation in skeletal muscle and adipose tissue are clearly different. These tissue specific differences could also be due to skeletal muscle and its vasculature being physically trained during RT, whereas adipose tissue is "passively trained". Thirdly, the fat loss (albeit small) in the current study may not have caused concomitant microvascular remodelling. It is well known that during adipose tissue expansion (hypertrophy) capillary density declines [370] and therefore reducing adipocyte size may not necessarily increase capillary density. Fourthly, it was observed that following six weeks of RT, circulating TNFα and sVCAM-1 levels were significantly elevated (Figure 6.3). It is well established that high intensity or RT training can promote an acute pro-inflammatory state [325, 371]. This pro-inflammatory phenotype in the T2D participants occurred despite avoiding exercise (including the RT program) for 48 hrs to returning to the clinic for cardio-metabolic testing. It has previously been demonstrated that the pro-inflammatory cytokine TNFα can cause skeletal muscle microvascular insulin resistance in healthy rats [372]. Whether the elevated TNFα and sVCAM-1 levels observed post-RT caused microvascular insulin resistance in adipose tissue is not known and warrants further investigation. In addition, our study focused on subcutaneous adipose tissue. Whether central (visceral) adipose tissue also displays the same microvascular abnormalities before and after RT is yet to be confirmed. It is known that adipose tissue blood flow increased during exercise in healthy people [251], so it is possible that six-week RT might not have changed baseline adipose tissue blood flow, but might still have improved adipose tissue blood flow during exercise.

In summary, the current chapter demonstrates that improvements in insulin sensitivity, glycaemic regulation, circulating lipids and body composition in people with T2D following six weeks of RT are not conditionally linked to improvements in MBV and MBF in adipose tissue. A pro-inflammatory phenotype after exercise training did not explain the lack of adipose microvascular improvements with RT. Improving microvascular function in adipose tissue of people with T2D as a novel approach to

prevent pathogenesis of obesity related complications such as IR, dyslipidaemia and glucotoxicity is uncertain.

Chapter 7: Study Significance and Future Directions

7.1 Key findings

Microvascular blood flow is important to deliver key nutrients (e.g. oxygen, lipids and glucose) and hormones to (e.g. insulin) and remove waste products from adipose tissue. Total ATBF increases after a meal, and this response is impaired in obesity and T2D. Microvascular blood flow is more important than total blood flow for nutrient exchange in many tissues, however it was not known whether microvascular blood flow in adipose tissue is altered by meals or T2D. Chronic exercise training improves microvascular blood flow in skeletal muscle of people with T2D. Whether adipose tissue microvascular responses are similarly improved following exercise training in people with T2D was unknown. The overarching goal of the current thesis was to characterise microvascular ATBF responses to a meal in healthy and T2D subjects, and determine whether these responses are altered by chronic exercise training.

The first aim characterised adipose tissue microvascular blood flow responses in the post-prandial state in healthy people. Adipose tissue microvascular blood flow was measured by contrast-enhanced ultrasound (CEU) at baseline and 1-hour after a mixed meal challenge or an oral glucose challenge (OGC). Adipose tissue MBV and MBF increased to a similar extent with both challenges. This increased microvascular perfusion of adipose tissue may improve delivery of key nutrients (e.g. glucose and lipid) from the meal for storage in adipose tissue.

The second aim investigated whether people with T2D have an impairment in adipose tissue microvascular responsiveness following an OGC, and whether systemic inflammation or the metabolic syndrome is associated with an adipose tissue microvascular-linked phenotype. Adipose tissue MBV and MBF post-OGC were markedly impaired in T2D when compared to healthy controls. These impaired microvascular responses in adipose tissue were associated with obesity, insulin resistance, hyperglycaemia and dyslipidaemia, but not systemic inflammation.

The final aim determined whether chronic exercise training restores adipose tissue microvascular blood flow in people with T2D. Adipose tissue microvascular blood flow

was measured by CEU before and after six-weeks (3 days per week) of a fully supervised resistance training program. Insulin sensitivity, glycaemic regulation, circulating lipids and body composition were all improved in people with T2D following resistance training. However, these favourable cardio-metabolic outcomes were not associated with a paralleled improvement in adipose tissue MBV and/or MBF.

Collectively, this thesis has demonstrated that a mixed meal or an OGC induces both MBF and MBV increases in adipose tissue in healthy but not people with T2D, and these impairments are not restored by six weeks of exercise training. The dissociation of impaired adipose microvascular blood flow from inflammation, but association with body fat, glycaemic response and lipid handling provides clues about the role of adipose tissue microvascular blood flow in metabolic derangements associated with T2D. In particular, changes in adipose tissue microvascular blood flow in obesity/T2D may affect lipid deposition prior to altering adipose tissue hypoxia and inflammation.

7.2 Summary of findings

7.2.1 Adipose tissue microvascular blood flow increases in the post-prandial state in healthy people

Chapter 3 demonstrated that glucose and insulin levels rose significantly following the ingestion of a MMC in healthy people. Adipose MBV significantly elevated 1-hr following the MMC when compare to baseline. Adipose tissue MBF was also higher compared to baseline but was borderline significant. This microvascular action may improve delivery of hormones (e.g. insulin) and nutrients (e.g. glucose and lipids) from the MMC to adipose tissue for storage.

Baseline adipose tissue MBV was negatively associated with fasting insulin, but positively associated with QUICKI (a surrogate marker of insulin sensitivity). Adipose tissue MBV following the MMC was positively associated with diastolic blood pressure (DBP). Also, baseline MBF was negatively associated with total body fat (%) and truncal fat (%). This suggested that MBF in adipose tissue is lower in people with obesity.

7.2.2 Adipose tissue microvascular blood flow increases in healthy people regardless of macronutrient profile

Chapters 3 and 4 compared the adipose tissue microvascular responses to an OGC and a MMC in healthy subjects. Compared with the OGC (which only contains glucose), the MMC contains protein, fat, and carbohydrate (including sugars). Blood glucose levels were significantly higher after the OGC when compared with the MMC, but both MMC and OGC produced a similar plasma insulin response. Importantly, the OGC and MMC produced a similar increase in MBV and MBF in adipose tissue of healthy people. This study has established for the first time in humans that adipose tissue microvascular responses are similar between the MMC and OGC despite very different macronutrient profiles and glycaemic loads. Therefore insulin, rather than the composition of the meal, may be a key regulator of adipose tissue microvascular blood flow.

7.2.3 Adipose tissue microvascular blood flow is impaired in people with T2D

In chapter 5, the difference in adipose tissue microvascular responses to the OGC was compared between people with T2D and healthy subjects. People with T2D had significantly higher body weight, BMI, total body fat (%) , trunk fat (%), fasting blood glucose, fasting plasma insulin, HbA1c, HOMA-IR, SBP, DBP, serum triglyceride and FFAs, and lower HDL and QUICKI when compared with control participants. Blood glucose levels in people with T2D were significantly higher than controls at every time point during the OGC. The glucose AUC over the 2hrs in the T2D people was significantly higher compared with controls. While fasting insulin levels were higher in the people with T2D, there were no differences in the time-course of plasma insulin levels during the OGC. As such, the insulin AUC for control subjects and those with T2D were also similar. However, there were no statistically significant differences observed in TNF-α, IL-6, CRP, MCP-1, IL-1β or sVCAM-1 between control subjects and those with T2D.

Chapter 4 demonstrated that adipose tissue MBV and MBF were elevated in the post-prandial state of healthy subjects. However, adipose tissue MBV in controls was significantly elevated 1 hr after the OGC and this response was completely absent in the people with T2D, being significantly lower than controls at the same time point.

Baseline MBF in T2D appeared lower than the control group and was significantly lower than controls 1hr into the OGC. This suggested that people with T2D have impaired adipose tissue MBV and MBF in response to an OGC compared to healthy subjects.

7.2.4 Adipose tissue microvascular blood flow is linked to the metabolic syndrome

Correlations were conducted to determine associations with adipose tissue microvascular responses (MBV and MBF) for healthy and T2D subjects. In Chapter 3, the degree of obesity was found to be a strong modifier of adipose tissue microvascular blood flow. In chapter 4, baseline MBF was also negatively associated with total body fat and truncal fat regardless of whether the group were allocated to MMC or OGC. Therefore, the greater degree of obesity similarly affects the ability to increase adipose tissue microvascular blood flow.

In chapter 5, fasting blood glucose, glucose AUC during the OGC, HbA1c, fasting insulin, TG and FFA levels were negatively associated with baseline and/or OGC MBF. QUICKI (a surrogate marker of insulin sensitivity) correlated positively with MBF. QUICKI was the only metabolic variable that correlated with MBV, being positively associated with the MBV response to the OGC. Fasting TG and FFA levels correlated negatively with MBF but not MBV. However most of these correlations disappeared in healthy people, however a negative correlation was observed between body fat and MBF in the healthy group (Chapter 4). Systemic inflammation was not associated with adipose tissue microvascular responses. This suggested that impaired microvascular function in adipose tissue was associated with obesity, insulin resistance, hyperglycaemia and dyslipidaemia, but not conditionally linked to systemic inflammation

7.2.5 Resistance training does not improve adipose tissue microvascular blood flow in T2D people.

In chapter 5, individuals with T2D had impaired adipose tissue MBV and MBF in response to an OGC compared to healthy subjects. Also, previous studies have shown resistance training improves skeletal muscle microvascular flow and consequently

metabolic function [299]. So, the aim of Chapter 6 was to determine if six weeks of RT augments adipose tissue microvascular responses in sedentary people with T2D and whether this is paralleled by improvements in IR, hyperglycaemia and dyslipidaemia.

In chapter 6, six-weeks of RT significantly improved metabolic function and reduced body fat, but did not improve adipose tissue microvascular blood flow in people with T2D. Six weeks of RT significantly reduced fasting blood glucose, HbA1c, glucose AUC and HOMA-IR. This was accompanied by a small but significant reduction in total body fat, trunk fat and fasting serum triglyceride levels. However, there were no significant changes in adipose tissue microvascular blood flow or volume at baseline or in response to OGC following RT.

7.2.6 Difference between microvascular responses in adipose tissue and skeletal muscle

In chapter 4, it was demonstrated that the OGC and MMC produced a similar increase in MBV and MBF in adipose tissue of healthy people. However, work by Russell and colleagues have shown that the OGC impaired microvascular responses (both MBV and MBF) in skeletal muscle of healthy individuals whereas the MMC was stimulatory on MBV and MBF [373]. This may suggest that the mechanism of stimulating blood flow in skeletal muscle and adipose tissue is different. Indeed, the mechanism of stimulating skeletal muscle blood flow is via insulin causing NO release from the endothelium via endothelial insulin receptor, stimulation and Akt activation of eNOS [349, 350]. The mechanism of regulating ATBF is complicated. NO blockade blocks the ATBF response to oral glucose loading [247], and others [273, 276] have shown that ATBF increases in response to insulin infusion. However, increases in adipose tissue blood flow in response to infusion of insulin is lower than that of oral glucose loading, suggesting additional vasodilatory signals in adipose tissue are involved [273]. Notably, β adrenergic receptor antagonism partially blocks the ATBF response to oral glucose, suggesting possible sympathetic nervous involvement in the vasodilation [247].

Moreover, chapter 6 demonstrated that improvements in insulin sensitivity, glycaemic regulation, circulating lipids and body composition in people with T2D following six weeks of RT are not conditionally linked to improvements in MBV and MBF in adipose

tissue. However, Russell et al have recently demonstrated that in addition to these cardiometabolic benefits such as improvements in glucose and HbA1c levels, six weeks of RT markedly enhances skeletal muscle MBF in T2D subjects in responses to an OGC [299]. One possible explanation is that regulation of the microcirculation between skeletal muscle and adipose tissue are different. Also, these tissue specific differences could also be due to skeletal muscle and its vasculature being physically trained during RT, whereas adipose tissue is "passively trained".

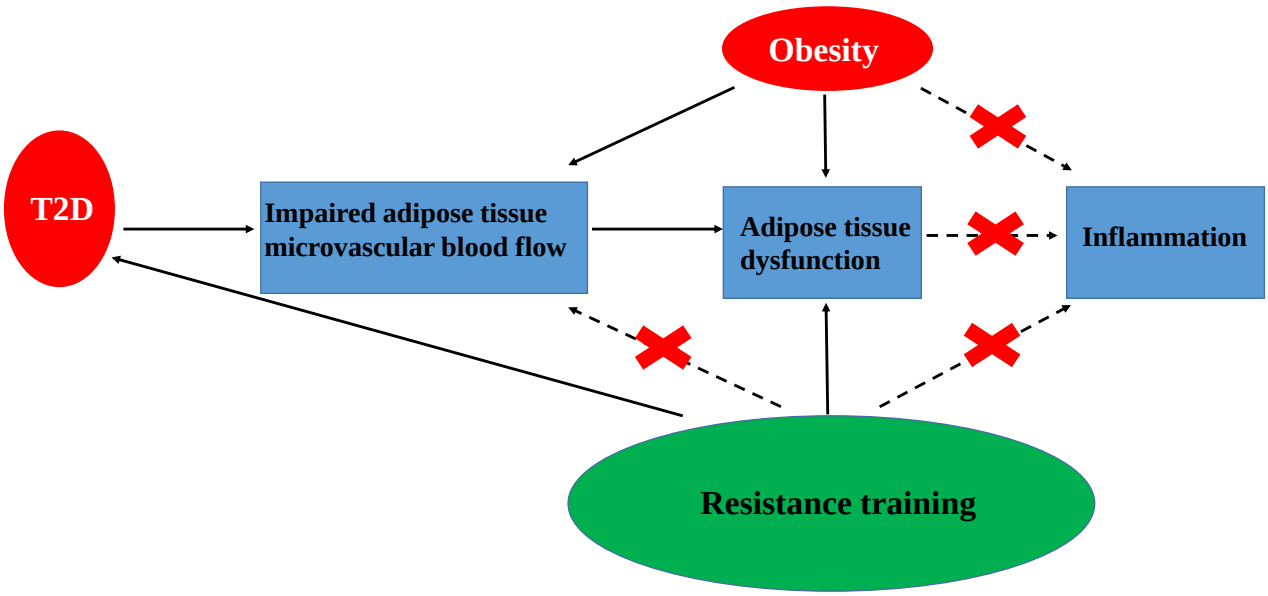

Figure 7.1: Main findings of the current project. Compared with healthy subjects, people with T2D had impaired microvascular ATBF and adipose tissue dysfunction, but do not display a pro-inflammatory state. Obesity was strongly associated with impaired microvascular ATBF and adipose tissue dysfunction, but this impaired blood flow was not linked with inflammation. Also, RT didn't improve adipose tissue microvascular blood flow and reduce inflammation, but it significantly reduced body fat and improved insulin resistance, hyperglycaemia and dyslipidaemia. Red indicates an impairment, green indicates an improvement.

7.3 Clinical implications

There are very few studies investigating microvascular responses in adipose tissue in humans. Those who have assessed adipose tissue microvascular responses have focused

on microvascular blood volume (MBV) and not microvascular blood flow (MBF). This project has demonstrated that both adipose tissue MBV and MBF increased to a similar extent with an oral glucose challenge and a mixed meal challenge. This increased microvascular perfusion of adipose tissue may improve delivery of key nutrients (e.g. glucose and lipid) from the meal for storage in adipose tissue. The possible clinical implications of this study could help to understand the role of adipose tissue blood flow on nutrient exchange and cardio-metabolic health.

Moreover, compared with controls, there is an impairment in both MBV and MBF in adipose tissue of people with T2D. The degree of obesity (independent of T2D) is a strong modifier of adipose tissue microvascular blood flow. Therefore, chronic exercise training was explored to determine whether improve adipose tissue MBV and MBF in people with T2D is linked to better metabolic health. Improvements in insulin sensitivity, glycaemic regulation, circulating lipids and body composition in people with T2D following six weeks of RT, but these effects are not conditionally linked to improvements in MBV and MBF in adipose tissue. As for the clinical complications of this study, improving microvascular function in adipose tissue may be a novel approach to prevent pathogenesis of obesity related complications such as IR, dyslipidemia and glucotoxicity in the future.

7.4 Limitations of this project

A limitation of the study was number of participants. The study should be performed with a larger number of participants to make sure similar results are reproduced. This would ensure results and significant correlations in healthy and T2D people found in the present study were not due to chance. Another limitation of the study was that the T2D participants were on a variety of medications when compared to the healthy controls. Although all diabetes related medications were omitted for 48hrs prior to attending the clinic for testing, participants were still taking other medications (e.g. statins and anti-hypertensives) which may have contributed, at least in part, to variations in inflammation and blood flow responses between healthy and T2D subjects.

Moreover, another limitation of the study was the temperature of clinic room. All patients were tested in the same clinical room with similar temperature settings, but no

temperature readings were taken. Thermoregulation may play a role in the adipose tissue microvascular perfusion.

Adipose tissue levels of pro-inflammatory markers were not measured, and histology was not conducted on adipose tissue to assess the degree of macrophage recruitment and activation, or assess adipose tissue oxygenation. In regards to the effect of RT on adipose tissue microvascular blood flow study, a limitation of this study was the lack of a non-exercising control group. The researcher was also not blinded during the analysis. This could potentially introduce a reporting bias which should be removed in future studies.

7.5 Future directions

The current thesis demonstrated that the OGC and the MMC produced a similar increase in MBV and MBF in adipose tissue of healthy people. However, the mechanism of the increase in MBV and MBF in adipose tissue is not fully understood. Further studies should investigate how and why the increase in adipose tissue MBV and MBF occurs.

Furthermore, people with T2D had impaired adipose tissue microvascular blood flow and they were unable to increase their MBV and MBF in response to an OGC. As these novel findings were limited to the subcutaneous central adipose tissue, future studies should explore the microvascular responses in visceral adipose depot (adipose tissue surrounding the abdominal organs).

Studies have suggested that the adipocyte undergoes hypertrophy, insufficient microvascular blood supply to the adipocyte leads to hypoxia, macrophage recruitment and conversion of macrophages from an inactive state (M2) to an active state (M1) in which they are reported to release inflammatory cytokines [85, 127]. However the current thesis did not observe higher inflammatory cytokines or a microvascular-linked pro-inflammatory condition in the T2D cohort tested. Further studies need to be conducted to measure adipose tissue levels of pro-inflammatory markers, or conduct histology on adipose tissue to assess the degree of macrophage recruitment and activation, or assess adipose tissue oxygenation.

Regarding adipose tissue function as a whole, there are several key questions to be answered in the future: (1) What is the relationship between insulin resistance and impaired adipose tissue microvascular blood flow? (2) Which one occurs first, insulin resistance or impaired adipose tissue microvascular blood flow? (3) What is the primary cause of reduced adipose tissue microvascular blood flow response? (4) What factors/treatments can improve adipose tissue microvascular blood flow in people with T2D? Further animal or clinical studies are essential to clarify these issues.

There were no significant improvements in adipose tissue microvascular responses in adipose tissue of people with T2D following RT. In the future, the length of training should be extended beyond 6 weeks to cause sufficient fat loss to see if improvements in adipose tissue MBV or can be observed. Also, further studies should investigate aerobic exercise training on adipose tissue microvascular blood flow due to its increased need for oxygen (and therefore, increased blood flow) and ability to decrease adipose tissue mass.

7.6 Conclusions

● Insulin is a key regulator in adipose tissue microvascular blood flow.

● Impaired microvascular function in adipose tissue during T2D is not conditionally linked to systemic inflammation, but is associated with other characteristics of the metabolic syndrome (obesity, insulin-resistance, hyperglycaemia and dyslipidaemia).

● Six-week RT does not improve adipose tissue microvascular blood flow of people with T2D, however it does significantly improve metabolic function and reduce body fat. Inflammation does not play a major role in regulating adipose tissue microvascular blood flow.

● Understanding the primary mechanism leading to poor adipose tissue microvascular blood flow and how to improve adipose tissue microvascular blood flow in people with T2D warrants further investigation. Improving adipose tissue microvascular blood flow

might be a new therapeutic target to improve management and co-morbidities associated with T2D.

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